2012

Microzooplankton Community Structure and Grazing Impact along the Western Antarctic Peninsula

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MICROZOOPLANKTON COMMUNITY STRUCTURE AND GRAZING IMPACT ALONG THE WESTERN ANTARCTIC PENINSULA

A Thesis

Presented to

The Faculty of the School of Marine Science

The College of William and Mary in Virginia

In Partial Fulfillment

of the Requirements for the Degree of

Master of Science

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by

Lori Michelle Price

2012

APPROVAL SHEET

This thesis is submitted in partial fulfillment of

the requirements for the degree of

Master of Science

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TABLE OF CONTENTS

ACKNOWLEDGEMENTS

I am very appreciative of the help and support of many friends and colleagues throughout my research and graduate studies. First I would like to thank my advisor, Dr. Debbie Steinberg, for her support and guidance during my studies at VIMS, challenging me to become a better scientist and writer, her enthusiasm and excitement for my research, and her moral support. Thanks to my committee members: Drs. Debbie Bronk, Walker Smith, Kam Tang, and Diane Stoecker for their valuable feedback and interest in my research.

I completed my research largely due to the support of the Principal Investigators and participants of the Palmer Antarctica Long-Term Ecological Research (PAL LTER) project, as well as the technical support of the captain and crew of the R/V *Laurence M. Gould* and the Raytheon Polar Services Group, both on the *L.M. Gould* and at Palmer Station. Your excitement and love for Antarctic research was apparent and I sincerely appreciate the hard work, dedication, and humor during the long hours working on the cruises and at Palmer Station. A special thanks to Dr. Hugh Ducklow for his help with my research and dedication to the PAL LTER project.

Thanks to members of my lab for helping during my experiments, lugging carboys of water, setting up the experiments and filtering for hours, including Joe Cope, Kate Ruck, Kim Bernard, and especially my undergraduate helpers Sarah Giltz, Caitlin Smoot, and Frances Armstrong. I couldn't have done those experiments without you. Also thanks to the other members of my lab for moral support, for visiting me in my "cave", and for your friendship: Josh Stone, Brandon Conroy, Miram Gleiber, and Jeanna Hudson. A special thanks to Josh Stone for his patience with me whilst helping me with GIS. Matthew Erickson spent countless hours working with me on the flow cytometer – you are a wonderful teacher and I appreciate your willingness to help me with all aspects of my research. Thanks to many other people who have helped me with my research and studies including Wayne Coats, Tsetso Bachvaroff, Mirko Lunau, Patrice Mason, and especially Dr. Grace Saba, who introduced me to the world of zooplankton.

Thanks to my friends for their love, support, and laughter through my graduate career, especially Emily Loose for her constant friendship. I never would have made it through graduate school without our homemade pizzas, sushi dinners, conversations, and time spent volunteering at the shelter. Also, to my lab mates, you were my family away from home and I can't imagine my time at VIMS without our good-natured sibling-like arguments, our lab dinners, and our hours spent together in the lab. I also want to thank my high school biology teacher, Dennis DiSilvio, for sparking my love of science.

Finally, and most importantly, I would not be where I am today without the love and support of my family. Mom and Dad, thank you for always encouraging me, pushing me to work harder, being proud of my accomplishments, and helping me achieve my dreams. Katie and Julie, you are not only my big sisters, but my best friends. Thank you for always being there. And finally, my fiancé Michael, you have been my rock and my emotional support, always reminding me of the important things in life. Thank you for your unconditional love and unwavering support.

LIST OF TABLES

LIST OF FIGURES

Chapter 3 Page

LIST OF APPENDICES

ABSTRACT

Microzooplankton (zooplankton 20 – 200 μm) are an integral part of aquatic food webs as they can be significant grazers of phytoplankton and bacteria, remineralizers of nutrients, and prey for higher trophic levels. The importance of microzooplankton in many pelagic ecosystems has been established, yet compared to larger zooplankton, microzooplankton are understudied in the Southern Ocean. I quantified microzooplankton community composition and grazing rates along the Western Antarctic Peninsula (WAP) as part of the Palmer Antarctica Long-Term Ecological Research project (PAL LTER). This region is experiencing rapid warming, causing declines in sea ice and changes in the marine food web. I determined distributions of major microzooplankton taxa in January 2010 and 2011 within the top 100 m of the water column, along both north-south and coastal-offshore gradients of the WAP, using microscopy. I found that microzooplankton are potentially adjusting to climate changes along with other trophic levels, as there was generally higher microzooplankton biomass in the south compared to the north. Biomass was higher in surface waters compared to 100 m, and variability in microzooplankton biomass between years and with distance from shore appeared to be influenced by sea ice dynamics. Microzooplankton biomass was also positively correlated with chlorophyll-*a* and particulate organic carbon (POC), and biomass of several microzooplankton taxonomic groups peaked near Marguerite Bay, historically a productivity hot spot. I also calculated phytoplankton and bacterial growth and grazing mortality rates using the dilution method at select stations along the WAP in January 2009 – 2011 and in the near shore waters near Palmer Station in February – March 2011. Microzooplankton exerted higher grazing pressure on bacteria, relative to grazing on phytoplankton. Microzooplankton also exhibited selective grazing on smaller phytoplankton (picoautotrophs and nanophytoplankton), and on the more actively growing bacterial cells, thus shaping phytoplankton and bacterial assemblages and effectively cropping production. There was a significant (albeit weak) positive correlation between temperature and phytoplankton grazing mortality. My research is the first to describe both the microzooplankton structure and grazing impact in the PAL LTER study region. This study contributes valuable information to studies modeling the flow of carbon through the WAP food web and provides a reference point for studying how future changes will affect microzooplankton community structure and food web dynamics in this region.

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MICROZOOPLANKTON COMMUNITY STRUCTURE AND GRAZING IMPACT ALONG THE WESTERN ANTARCTIC PENINSULA

CHAPTER 1

Introduction

Climate change in the Western Antarctic Peninsula

The Western Antarctic Peninsula (WAP) is currently experiencing one of the fastest rates of warming seen on Earth, with a 6°C increase in average winter air temperature since 1950 (Smith et al. 1996, Vaughan et al. 2003). This has led to an increase in the ocean heat content over the continental shelf (Martinson et al. 2008). The warming and upwelling of Upper Circumpolar Deep Water onto the WAP continental shelf is the main mechanism driving sea ice and glacial melt (Ducklow et al. 2012). This warming shelf water, coupled with changes in atmospheric circulation patterns, has also led to a decrease in sea ice extent and a change in the timing of sea ice advance and retreat. A shorter sea ice season has now been documented for the WAP, with 30 – 40 fewer days of ice cover during the period from 1992-2004 relative to ice cover from the previous decade (Stammerjohn et al. 2008).

Sea ice dynamics are a fundamental component of Antarctic marine ecosystems, affecting everything from the timing and scale of primary production to the reproductive success of krill and higher-level predators (Quetin et al. 1996, Chapman et al. 2004, Stammerjohn et al. 2008, Vernet et al. 2008). For example, reduced sea ice cover and stronger winds in the northern WAP have led to a decrease in phytoplankton biomass, while declining sea ice in the southern WAP has opened more habitat for phytoplankton growth in a region once permanently ice-covered, leading to an increase in phytoplankton biomass in the south (Montes-Hugo et al. 2009). Antarctic krill (e.g., *Euphausia superba*), a key component of some Antarctic marine food webs and important prey for higher predators (e.g. penguins, seals, and whales) (Laws 1985), depend on sea ice for critical aspects of their life history (Quetin et al. 1996). Declines in krill stocks coincident

with decreases in sea ice have been noted (Atkinson et al. 2004), which will affect higher-level predators that depend on them. It is unknown if climate-induced changes in the WAP food web are reflected in microzooplankton distributions, as microzooplankton are understudied in this region compared to meso- and macrozooplankton; no baseline of their abundance and distribution is available. Microzooplankton are a key component of aquatic food webs (Sherr and Sherr 2002, Calbet and Landry 2004), and understanding their distribution and trophic role is essential for a complete understanding of the structure and function of the WAP food web, as well as for predicting future climate-induced change in trophic dynamics.

Importance of microzooplankton in aquatic food webs

Microzooplankton (zooplankton 20 – 200 μm) include protozooplankton and tiny metazoans such as larval stages of certain organisms (e.g., copepod nauplii). They are abundant and found in all aquatic environments worldwide (Sherr and Sherr 2002) The majority of microzooplankton are heterotrophic protozooplankton (unicellular, eukaryotic organisms) that are comprised of heterotrophic flagellates, ciliates, naked amoebae, foraminiferans, acantharians, and radiolarians (Stoecker and Capuzzo 1990). Microzooplankton can be significant consumers of bacteria, phytoplankton, other protists and metazoan eggs, can be a significant food source for metazoans, and are important remineralizers of nutrients (e.g., nitrogen, phosphorus, and trace metals) (Sherr and Sherr 2002). Mixotrophy is also common among protists (Stoecker 1999, Fenchel 2008). Due to a variety of feeding strategies (Hansen and Calado 1999, Jeong 1999, Tillmann 2004), micrograzers can consume a wide range of prey types and sizes (Sherr and Sherr 2002)

and, in better-studied non-polar waters are known to influence phytoplankton and bacterial assemblages due to selective feeding (Stoecker 1988, Banse 1992, Sherr and Sherr 1994).

In addition to shaping phytoplankton assemblages, micrograzers can also exert significant grazing pressure on phytoplankton. In a review of microzooplankton grazing rates worldwide, Calbet and Landry (2004) concluded that microzooplankton are responsible for consuming 59-77% of primary production across a wide range of environments and are the main source of phytoplankton mortality in the sea. This is partially because micrograzers have growth rates comparable to their prey and can readily respond to fluctuations in food supply, relative to the longer generation and response times of metazoans (Sherr and Sherr 2002, Tillmann 2004, Calbet 2008).

In addition to their importance as herbivores, microzooplankton (particularly heterotrophic nanoflagellates) are key consumers of bacterioplankton. Flagellate populations can graze a high proportion of the measured daily production of bacterioplankton (Sherr and Sherr 1994) and can alter bacterial assemblage structure by selective feeding (Sherr et al. 1992, Sherr and Sherr 1994). Bacteria in coastal Antarctic waters ultimately depend on phytoplankton production for organic carbon and therefore should be coupled with phytoplankton populations. Summer bacterial abundances are relatively constant along the WAP (Ducklow et al. 2012). High microzooplankton grazing pressure could effectively crop bacterial biomass, helping explain the relatively constant bacterial abundances in this region.

The role of microzooplankton in Southern Ocean food webs

The Antarctic food web was historically viewed as short and efficient with large diatoms efficiently grazed by krill (El-Sayed 1971) which are then directly consumed by predators such as penguins, seals, and baleen whales (Ducklow et al. 2007, 2012). The roles of smaller primary producers (e.g. pico- and nanophytoplankton, 0.2-20 μm) and consumers (e.g. heterotrophic protozooplankton, 2-200 μm) (Porter et al. 1985) in the Antarctic food web were largely ignored (von Brockel 1981, Garrison 1991) until the late-1970s and 80s, when new evidence emerged of the importance of autotrophic nanoplankton and protozoan consumers in the Antarctic (von Brockel 1981, Buck and Garrison 1983, Hewes et al. 1985, Heinbokel and Coats 1986). Microzooplankton are also an important component of meso-and macrozooplankton diets in the Southern Ocean (Atkinson and Snÿder 1997, Lonsdale et al. 2000, Schmidt et al. 2006), providing an important link between these larger consumers and small primary producers and bacteria. Despite this evidence, there have been relatively few studies of protozooplankton, compared to meso- and macrozooplankton (i.e. >200 μm) in Antarctic food webs.

Microzooplankton can be as abundant in the waters surrounding Antarctica as they are in other regions of the world, though they have extremely patchy distributions (Garrison 1991, Umani et al. 1998, Landry et al 2002). Microzooplankton grazing rates also vary seasonally and spatially with respect to total grazing impact on primary producers, with published rates ranging from 0 – 87% of the phytoplankton standing stock removed per day and 0 – >100% of the potential primary production removed per day (Garrison 1991, Caron et al. 2000, Pearce et al. 2010). Despite the wide range of values reported for microzooplankton grazing in the Southern Ocean, a balance between

phytoplankton growth and microzooplankton grazing has been observed, suggesting strong coupling in certain regions at specific times (Burkill et al. 1995, Pearce et al. 2008). Alternatively, large phytoplankton blooms at times suggest a decoupling between phytoplankton and grazers (Tagliabue and Arrigo 2003, Rose and Caron 2007), highlighting the complexity of these interactions. These results emphasize the potential importance of microzooplankton as a component of Southern Ocean food webs.

Goals and structure of thesis

My thesis explores microzooplankton community composition and grazing impact along the WAP. The results constitute the most comprehensive picture to date of the role microzooplankton play in the structure and functioning of the WAP microbial food web, and provide a reference point for future studies of climate-induced changes in the food web of this region. In this thesis, the term "microzooplankton community" refers to all taxonomic groups of microzooplankton (zooplankton 20 – 200 μm) and heterotrophic protozooplankton (unicellular, eukaryotic organisms) occupying a specific habitat and comprising a variety of trophic levels.

This thesis is separated into two chapters, each part addressing specific research questions. Chapter 2 presents results from field sampling and describes the microzooplankton community composition along the WAP. I quantified the major microzooplankton taxonomic groups, and investigated how microzooplankton changed along both a north-to-south gradient, as well as with distance from shore and with depth. I determined if microzooplankton biomass was correlated with chlorophyll-*a* and particulate organic carbon, determined regions of high microzooplankton biomass, and looked at the effects of bottom-up controls on microzooplankton distributions.

Chapter 3 presents results from field experiments examining the potential grazing impact of microzooplankton on natural assemblages of phytoplankton and bacteria. I quantified microzooplankton grazing rates using the dilution method, and was able to compare my results with previously published studies that determined grazing by macrozooplankton. I determined that microzooplankton demonstrated selective feeding on different size classes of phytoplankton or different bacterial types, and also assessed the effects of physical forcing (e.g., storm events) and the environment (e.g., water temperature) on microzooplankton grazing rates.

Finally, Chapter 4 concludes the thesis with a summary and implications of my results and provides suggestions for future research.

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CHAPTER 2

Microzooplankton community composition along the Western Antarctic Peninsula

ABSTRACT

Microzooplankton are an integral part of aquatic food webs, yet compared to macrozooplankton, are understudied in the Southern Ocean. The region along the Western Antarctic Peninsula (WAP) is experiencing rapid climate warming, resulting in declines in sea ice extent and duration, and affecting the marine food web. Microzooplankton community structure along the WAP was analyzed in January 2010 and 2011 as part of the Palmer Antarctica Long-Term Ecological Research project. Whole water samples were collected within the top 100 m of the water column along both north-south and coastal-offshore gradients, and major taxa of microzooplankton were quantified using microscopy. Average chlorophyll-*a* concentrations and microzooplankton biomass were higher in 2011 compared to 2010. Athecate dinoflagellates and aloricate ciliates dominated microzooplankton biomass, and the biomass of most taxonomic groups was higher in the south compared to the north. Specifically, aloricate ciliate and tintinnid biomass increased with increasing latitude, and biomass peaked at several southern, inshore stations – including Marguerite Bay which was an area of high biomass for some microzooplankton taxa. Biomass was higher in surface waters compared to 100 m, and variability in microzooplankton biomass between years and with distance from shore was most likely due to sea ice dynamics. Microzooplankton biomass was positively correlated with chlorophyll-*a* and particulate organic carbon. These results suggest that microzooplankton populations may be adjusting to changes along the WAP.

INTRODUCTION

The importance of microzooplankton in marine food webs as grazers (Pace 1988, Calbet and Landry 2004), food for higher trophic levels (Stoecker and Capuzzo 1990, Fessenden and Cowles 1994, Froneman et al. 1996), and recyclers of nutrients (Andersen et al. 1986, Sherr and Sherr 2002) is now widely recognized. However, in the Southern Ocean where the majority of food web studies have focused on the abundance, distribution, and grazing of krill and other macrozooplankton (Quetin et al. 1996, Dubischar and Bathmann 1997, Pakhomov et al. 1997, Ross et al. 2008), the role of smaller consumers (e.g. heterotrophic protozoans <200 μm) is poorly defined. It is particularly important in an era of rapid environmental change to determine a reference point of species abundance and distributions, particularly in regions where such changes are likely to be pronounced (Smith et al. 1999).

The Western Antarctic Peninsula (WAP) is a region currently experiencing one of the fastest rates of warming on Earth, with an increase in average winter air temperature of approximately 1°C per decade since 1950 (Smith et al. 1996, Vaughan et al. 2003, Ducklow et al. 2012). Concurrently, the ocean heat content over the continental shelf in this region has steadily increased since 1993, due to the warming and upwelling of Upper Circumpolar Deep Water (UCDW) onto the shelf (Martinson et al. 2008). This is the primary mechanism for sea ice and glacial melt along the WAP (Ducklow et al. 2012), and, coupled with atmospheric variables, has caused a decrease in sea ice extent and a change in the timing of sea ice advance and retreat (Stammerjohn et al. 2008a). This regional warming has differentially affected the WAP, with a warmer, sub-Antarctic climate invading the northern part of the Peninsula and replacing the typical cold, dry

Antarctic climate still present in the southern part of the Peninsula (Smith et al. 1999, Ducklow et al. 2012). These contrasting climate regimes make the Antarctic Peninsula especially sensitive to climate change (Smith et al. 1999).

Phytoplankton have been responding to these long-term changes in air temperature and sea-ice dynamics along the WAP, and shifts in phytoplankton could affect the distribution and community composition of microzooplankton, as well as higher trophic levels that feed on them. Less sea ice cover and stronger winds (leading to deeper mixing and light limitation for phytoplankton) in the northern WAP has led to a decrease in chlorophyll concentrations and average phytoplankton size. In contrast, the decrease in sea ice in the southern WAP, which was previously permanently ice-covered, has released phytoplankton from light limitation and caused an increase in phytoplankton biomass (Montes-Hugo et al. 2009). This change in chlorophyll-*a* distribution along the WAP has been accompanied by shifts in phytoplankton composition, where assemblages in the north are dominated by smaller phytoplankton while those in the south have a greater fraction of diatoms and larger cells (Montes-Hugo et al. 2009).

In the Southern Ocean microzooplankton can be as abundant as they are in other regions of the world, and exhibit distinct seasonality and extreme patchiness (Garrison 1991a, Umani et al. 1998, Landry et al. 2002). Microzooplankton abundance is often correlated with chlorophyll-*a* and particulate organic carbon (POC), indicating an influence of food supply on their distribution (Heinbokel and Coats 1986, Burkill et al. 1995, Archer et al. 1996, Becquevort 1997, Klaas 2001). Studies quantifying microzooplankton structure in the Southern Ocean indicate that the dominant microzooplankton can change dramatically along a transect (Alder and Boltovskoy

1993), with some changes associated with different water masses (Burkill et al. 1995, Safi et al. 2007). Only two studies have described microzooplankton along the WAP. Calbet et al. (2005) found that the assemblage was dominated by aloricate ciliates, and that dinoflagellate abundance (represented by the genus *Gyrodinium*) was one order of magnitude lower than that of ciliates. Also, ciliate abundances peaked between 40 and 80 m and coincided with high ammonium concentrations, while dinoflagellates had a more homogeneous depth distribution (Calbet et al. 2005). Alder and Boltovskoy (1991) found that microzooplankton, especially tintinnids, appeared to be associated with sea ice along the WAP. Tintinnids and dinoflagellates were tightly correlated with each other, with highest abundances occurring in the southern Bellingshausen Sea near the coast.

This study provides an extensive description of microzooplankton community structure in the WAP along both north-south and coastal-offshore gradients, which is needed to better understand the factors that affect their abundance and distribution. Most importantly, these results provide a reference point for quantifying how future changes in this rapidly-warming region will affect microzooplankton community structure and WAP food web dynamics.

MATERIALS AND METHODS

Study site

As part of the Palmer Antarctica Long-Term Ecological Research (PAL LTER) project, microzooplankton samples were collected on annual research cruises aboard the R/V *Laurence M. Gould* in January 2010 and 2011. The PAL LTER sampling grid area along the WAP extends from Palmer Station on Anvers Island (64.77°S, 64.05°W) south to Charcot Island (69.45°S, 75.15°W) and from coastal waters to approximately 200 km offshore (Ducklow et al. 2007, 2012). Water samples for microzooplankton analysis were collected at select stations on this sampling grid (Figure 2.1).

Sample collection

For the purposes of this study, we only considered single-celled protozoa (i.e., heterotrophic dinoflagellates and ciliates) because tiny larval stages of metazoans (i.e., copepod nauplii), which are also considered microzooplankton (operationally defined as heterotrophic organisms 20 – 200 μm), were often much larger than 200 μm and not abundant. Thus the term 'microzooplankton' will hereafter be used to describe single-celled heterotrophic protists whose sizes ranged from 20 – 200 μm. A few microzooplankton groups were slightly smaller (e.g., some dinoflagellates) or larger (e.g., *Laackmaniella* spp., *Protoperidinium antarcticum*) and were included in this analysis. Larger, rarer protozooplankton (i.e., foraminifera, acantharia, and radiolaria), present in net-collected (64 μm mesh) samples in a companion study, are not considered.

To enumerate microzooplankton, whole water samples were collected using 12 L Niskin bottles mounted on a CTD rosette at three depths: surface (10 m), subsurface

chlorophyll-*a* maximum (as determined by *in situ* fluorescence, see Appendices 1 and 2 for depth), and deep (100 m). Fluorescence measured *in situ* was significantly positively correlated with extracted chlorophyll-*a* ($p<0.001$, data courtesy of O. Schofield and D.G. Martinson). For each depth, 200-250 ml whole seawater was preserved with acid Lugol's (final concentration 6 – 8%) and stored in the dark at room temperature up to 12 weeks or refrigerated at +4°C for up to one year until sample processing in our laboratory at the Virginia Institute of Marine Science. Although acid Lugol's (high concentrations, ~10%) is among the best solutions for preserving microzooplankton such as ciliates, cell losses still likely occurred (Stoecker et al. 1994).

Sample processing

Microzooplankton were identified to major taxon, but some abundant or conspicuous forms were identified to genus or species level. One sample per station and depth was counted. Acid Lugol's preserved samples were first concentrated by settling in the collection jars for at least one week and then removing the top half of supernatant. Aloricate ciliates, tintinnids, and the silicoflagellate *Dictyocha speculum* were enumerated by settling subsamples of the remaining half in 50 ml Utermöhl chambers, and dinoflagellates by settling subsamples in either 10 ml or 50 ml Utermöhl chambers. The entire contents (containing at least 100 cells, but typically 200 – 500 cells of the most abundant taxa) were counted using an inverted microscope after a minimum 24-hour settling period (Utermöhl 1931). The silicoflagellate *Dictyocha speculum* was included in the analysis because live silicoflagellate cells have been observed to have pseudopodia extending from their spines, indicating heterotrophy (Martini 1977). Dinoflagellates were

counted separately after clearing the sample with several drops of 5% sodium thiosulfate, and using the Utermöhl method described above. Heterotrophic nanoflagellates (<20 μm) were not enumerated as the Utermöhl method severely underestimates their abundance (Davis and Sieburth 1982).

Heterotrophic dinoflagellates were distinguished from autotrophic dinoflagellates using epifluorescence microscopy, both by spot-checking live samples shipboard, and by filtration and DAPI staining for analysis back at our home laboratory. Samples (20 – 50 ml) were preserved with glutaraldehyde (final concentration 1%) stained with 4'6' diamidino-2-phenylindole (DAPI), then subsequently filtered onto 5 μm Nuclepore polycarbonate black membrane filters under gentle vacuum filtration (<200 mm Hg) on ship. The filter was mounted on a slide and stored at -20°C until examination with epifluorescence microscopy (Sherr and Sherr 1993a, Sherr et al. 1993). However, because mixotrophy is common among protists (Fenchel 2008), creating divisions between heterotrophs and autotrophs is often irrelevant. In our study many dinoflagellates were binned into size classes (e.g., small 20 – 50 μm) rather than identified by genus or species, and some autotrophs/mixotrophs were most likely included in these analyses.

Biomass calculations

The biomass of each group was estimated by assigning standard geometric shapes or combinations of shapes to specific organisms and measuring the dimensions (Edler 1979, Hillebrand et al. 1999). Measurements were taken for at least 50 individuals for the abundant taxa and all present individuals for the rare taxa using Image Pro Plus 6.2 software (Media Cybernetics, Bethesda, MD). These volume (μm^3) measurements were

converted to estimates of carbon content (Table 2.1) by using the following conversion factors, dinoflagellates: pg C = volume$^{0.819}$ × 0.76 (Menden-Deuer and Lessard 2000); tintinnids: pg C = (volume × 0.053) + 444.5 (Verity and Lagdon 1984); other ciliates: pg C = volume × 0.19 (Putt and Stoecker 1989); all other groups: pg C = volume × 0.08 (Beers and Stewart 1970). These biomass calculations were were generally comparable to those calculated in the Ross Sea (Umani et al. 1998), although are likely underestimates as Lugol's preservative causes cell shrinkage (Stoecker et al. 1994).

Subdivision of LTER grid and statistical analyses

The LTER sampling grid was divided into sub-regions, (i) north and south, and (ii) inshore, midshelf, and offshore (Figure 2.1). To be consistent with previous LTER studies, these delineations are based on hydrographic and sea ice conditions (Martinson et al. 2008, Stammerjohn et al. 2008a, Bernard et al. 2012). Sea ice advance is earlier and retreat is later in the south, inshore compared to north, offshore. The north, inshore region is influenced by glacial melt, while the south has a larger influence of sea ice melt. The north has lower sea ice persistence and higher variability in sea ice cover compared to the south. The offshore region in the north is influenced by the Southern Antarctic Circumpolar Current Front while the south is influence by Upper Circumpolar Deep Water. The inshore-midshelf-offshore regions are also delineated by bathymetric features and are separated by temperature and salinity, where offshore waters are continuously influenced by the Antarctic Circumpolar Current (ACC). Inshore waters are rarely influenced by the ACC, and midshelf waters represent a transitional region (Martinson et al. 2008, Stammerjohn et al. 2008a). Additionally, the north-south demarcation is also

based on a change in penguin diet, an integral component of the PAL LTER project (Fraser et al. in prep).

Data were transformed using the natural log transformation to fit the normality and homogeneity of variance assumptions of the ANOVA. This was the best transformation as determined by the Box-Cox transformation procedure (Neter 1996). For comparison of upper water column microzooplankton community structure across the sampling grid, abundance and biomass data were integrated from 0 – 100 m. For this purpose we assumed that abundance and biomass at 0 m were equal to that measured at 10 m (no samples were collected at 0 m, but the mixed layer depth was always deeper than 10 m). For abundance and biomass values at each sampled depth, see Table 2.2. Statistical comparisons of the effect of latitude (north vs. south) and shelf location (distance from shore) on microzooplankton integrated biomass (0 – 100 m) were done using two-way ANOVA ($\alpha = 0.05$). The effect of depth on microzooplankton biomass was analyzed using one-way ANOVA ($\alpha = 0.05$).

A Bray-Curtis Index was calculated to examine the similarity of microzooplankton assemblages among locations (Bray and Curtis 1957, Bloom 1981), and cluster analyses based on those indices were created using BioDiversity Pro software (McAleece et al. 1997). Correlation between microzooplankton abundance and chlorophyll-*a* concentration and POC was determined by linear regression ($\alpha = 0.05$).

RESULTS

Hydrographic setting

Surface water temperatures at the stations sampled ranged from -1.3 to 1.0°C (mean = 0.1°C) in 2010 and were significantly higher in 2011 ($p<0.001$), ranging from -0.8 to 2.1°C (mean = 1.0°C). Surface salinities ranged from 32.7 to 34.0 (mean = 33.7) in 2010 and 32.6 to 33.9 (mean = 33.5) in 2011, and there was no significant difference in salinity between years. Chlorophyll-*a* concentrations at the locations and depths where samples were collected for microzooplankton analyses ranged widely, from 0.005 to 8.51 μg L^{-1} in 2010 and 0.033 to 25.1 μg L^{-1} in 2011 (Appendix 1 and 2). The average chlorophyll-*a* concentration in 2011 (3.79 μg L^{-1}) was significantly higher ($p<0.01$) than in 2010 (1.23 μg L^{-1}). In 2011 most chlorophyll-*a* concentrations were <15 μg L^{-1}; however, a high value of 25.1 μg L^{-1} occurred near Marguerite Bay, historically a productivity hot-spot (data courtesy of H. Ducklow and O. Schofield) (Garibotti et al. 2003, Ducklow et al. 2012). See Appendices 1 and 2 for physical and biological data at each location/depth where microzooplankton samples were collected. Despite an overall decline in sea ice extent in the past 30 years (Stammerjohn et al. 2008a), sea ice extent in the WAP region was slightly higher in 2011 compared to 2010. Sea ice extent refers to the total area enclosed by the outer ice edge (concentrations ranging from 15-100%) and was 7.91×10^4 km^2 in 2010 and 9.37×10^4 km^2 in 2011 (data courtesy of S. Stammerjohn).

Microzooplankton distribution

Between-year and latitudinal comparison

Between-year variability in microzooplankton abundance and biomass was high (Figure 2.2, Table 2.2), but with few exceptions, athecate dinoflagellates and ciliates comprised the majority of the microzooplankton biomass in both years (Figures 2.2 and 2.3). Among most taxonomic groups, abundance and biomass were higher in 2011 compared to 2010, with the exception of an unidentified flagellate (Figure 2.4f-h) and the silicoflagellate *Dictyocha speculum* (Figure 2.2, Table 2.2). The unidentified flagellate was very abundant and contributed substantially to the total microzooplankton biomass in 2010, but was not as prevalent in 2011 (Figures 2.2 and 2.3). Preliminary genetic analyses indicate this organism could be fungal, but as it has not been positively identified it was included in these analyses. In general, abundance and biomass of most taxonomic groups (excluding the unidentified flagellate in both years, and the silicoflagellate in 2011) were higher in the south compared to the north. Trends in abundance and biomass were similar, and as biomass more accurately represents carbon and energy available to higher trophic levels, we report the remainder of our results as biomass.

Average ciliate and tintinnid biomass was significantly higher in the south compared to the north in 2010 and 2011 ($p<0.05$) (Figure 2.2c,d), and in general, increased with increasing latitude (Figure 2.5). In both years, biomass of many of the larger microzooplankton taxa, such as large tintinnids and athecate and thecate dinoflagellates (>60 μm in length/diameter), was higher in the south compared to the north (Student's t-test, $p < 0.05$). For example, both the thecate dinoflagellate

Protoperidinium antarcticum and the large tintinnid *Cymatocylis drygalskii* abundance and biomass were high in the south (Figure 2.6). In 2010 ciliate and tintinnid biomass both peaked at Station 4 in the south (Figure 2.5). At this station integrated (0-100m) chlorophyll-*a* and POC concentrations were moderate (Table 2.3). In 2011 ciliate biomass was highest at Station 3 at the southern end of Marguerite Bay (Figure 2.5a). This high ciliate biomass was mainly driven by a large bloom of the aloricate ciliate *Strobilidium* spp. At the chlorophyll-*a* maximum, small *Strobilidium* spp. (<30 μm) reached concentrations of 26,600 ind. L^{-1} (mean of all chl-*a* max. samples = 1,500 ind. L^{-1}), while large *Strobilidium* spp. (>30 μm) reached concentrations of 990 ind. L^{-1} (mean of all chl-*a* max. samples = 105 ind. L^{-1}). Chlorophyll-*a* concentrations were also moderate at this station (Table 2.3), but here we found the lowest salinity recorded in both years (32.7). While tintinnid biomass peaked even further south near Charcot Island (Station 5), the second highest record of tintinnid biomass was recorded at Station 3 at the southern end of Marguerite Bay (Figure 2.5b), where the highest ciliate biomass was in 2011 (Figure 2.5a).

The peaks in ciliate and tintinnid biomass in the north in 2011 occurred at Station 1 and 2, respectively (Figure 2.5), although chlorophyll-*a* and POC concentrations at these stations were relatively low (Table 2.3). Station 1 was located in the Palmer Deep Canyon and Station 2 was located in the north in the middle of the continental shelf (Figure 2.1). The high tintinnid biomass at Station 2 was a result of relatively high abundance of the small but abundant tintinnids *Salpingella* spp. and the large tintinnids *Laackmaniella* spp. (Appendix 3) at the chlorophyll-*a* maximum.

Trends with distance from shore and depth

There was also high between-year variability in biomass with distance from shore. For example, in 2010 aloricate ciliate biomass was higher offshore, whereas in 2011 their biomass was higher inshore near the coast. The unidentified flagellate biomass was considerably higher in the inshore and mid-shelf locations compared to offshore in 2010, but in 2011 there was no significant trend in their biomass with distance from shore (Figure 2.7). In 2011 biomass of most taxa was higher closer to shore, with the exception of the silicoflagellate (Figure 2.7b).

Trends in biomass with depth were consistent between years. Among most microzooplankton taxa, there was no statistical difference between biomass at 10 m depth and the chlorophyll-*a* maximum, but biomass was significantly lower at 100 m than at shallower depths (3 – 18 times lower; the unidentified flagellate in 2010 was 50 times lower at depth) (Figure 2.8). The only exception was for the tintinnids in 2010 for which there was no significant decrease in biomass at 100 m (Figure 2.8a).

Biological hot spots

Spatial contour plots of integrated microzooplankton biomass in 2011 illustrate the patchiness of microzooplankton distribution while also highlighting high biomass near Marguerite Bay for aloricate ciliates (Figure 2.9a), *Gyrodinium* sp., a large, conspicuous athecate dinoflagellate (Figure 2.9b), tintinnids (Figure 2.9c) and *Salpingella* spp. – a group of small but abundant tintinnids (Figure 2.9d). The higher ciliate biomass (~500 mg C m^{-2}) in the northern, inshore part of the sampling area was in the Palmer Deep Canyon (Figure 2.9a). The high tintinnid biomass in the south near Charcot Island

was driven by high abundance of the large tintinnid *Epiplocyloides antarctica* at the chlorophyll-*a* maximum (680 ind. L^{-1}, mean for the sampling grid = 60 ind. L^{-1}, Figure 2.9c). Higher food supply in biological hot spots (such as Marguerite Bay) could have influenced the distribution of microzooplankton, as total microzooplankton biomass was positively correlated with POC and chlorophyll-*a* (Figure 2.10a,b). POC samples were defined as all carbon collected on a 0.7 μm pore filter, including microzooplankton; however, microzooplankton only comprised approximately 10% or less of total POC (Figure 2.10a).

Marguerite Bay is located in the southern sub-region and high microzooplankton biomass here could have driven the higher microzooplankton abundance and biomass usually seen in the south compared to the north (Figure 2.2). When stations near Marguerite Bay (Station 3, and the three stations clustered just south of Adelaide Island at the mouth of Marguerite Bay, Figure 2.1) were removed from the analysis, microzooplankton abundance and biomass were still significantly higher in the south compared to the north. The only exceptions were for unidentified flagellate abundance in 2010, and tintinnid biomass in 2011, for which there was no statistical north-south difference with removal of Marguerite Bay stations ($p = 0.10$, Appendix 4).

Other regional groupings of taxa

The cluster analysis indicates that microzooplankton taxonomic composition and biomass for all stations were >64% similar in 2010 and >68% similar in 2011 (Figure 2.11), and there were few clear geographic trends to the clusters. When chlorophyll-*a* concentrations, water temperature, and salinity were removed from the cluster analysis,

the groupings did not change; therefore only microzooplankton taxonomic biomass was included in results presented here. Cluster A contained the majority of stations in 2010 as did Cluster D in 2011 (Figure 2.11). Cluster B included stations located in the northern, inshore region of the study area, and was characterized by low biomass of ciliates and absence of the ciliate *Didinium* sp. and tintinnids *Salpingella* spp. and *Laackmaniella* spp. (Figure 2.4b,c). Cluster A contained stations with higher ciliate biomass (especially large *Strobilidium* spp., Figure 2.4a) and tintinnid biomass, but did not contain exclusively southern stations.

In 2011 the largest cluster (D) was characterized by moderate microzooplankton biomass, and also low biomass of the large tintinnid *Cymatocylis drygalskii* and the large dinoflagellate *Protoperidinium antarcticum* (Figure 2.4e). This cluster included stations at all latitudes sampled and at all distances from shore. Stations in Cluster C were located near Marguerite Bay and had very high microzooplankton biomass, especially of the ciliate *Didinium* sp., the tintinnids *Laackmaniella* spp., athecate dinoflagellate *Gyrodinium* sp., and thecate dinoflagellate *Protoperidinium antarcticum* (Figure 2.4b-e), but had low biomass of the small ciliates *Strombidium* spp. Cluster E was comprised of stations with low tintinnid, athecate and thecate dinoflagellates, and silicoflagellate biomass. This cluster included the most northern inshore station, as well as stations in the south in the middle of the shelf.

DISCUSSION

Physical forcing and bottom-up control on microzooplankton distribution

The marine ecosystem of the WAP is seasonally productive and characterized by very large phytoplankton blooms (Smith et al. 2008, Vernet et al. 2008). Because macronutrients are usually high initially (Prézelin et al. 2004, Ducklow et al. 2012), light is a key factor limiting phytoplankton growth in this region. Phytoplankton biomass accumulates when mixed layer depths shoal, as a result of increased stratification due to sea ice melt and low wind speeds (Garibotti et al. 2005, Vernet et al. 2008, Montes-Hugo et al. 2009). This high phytoplankton biomass (and thereby food supply) can lead to high microzooplankton biomass (Heinbokel and Coats 1986, Alder and Boltovskoy 1993, Burkill et al. 1995, Archer et al. 1996, Becquevort 1997, Klaas 2001), as evidenced by the significant positive correlation between microzooplankton and chlorophyll-*a* and POC in this study (Figure 2.10).

Differences in chlorophyll-*a* concentration and microzooplankton biomass between 2010 and 2011 may in part be due to between-year differences in sea ice cover and water column stratification. Although most evidence has been circumstantial and the idea has been debated, in some circumstances phytoplankton blooms might be seeded by sea ice retreat (Garrison et al. 1987, Lizotte 2001, Leventer 2003). It is well documented that large phytoplankton blooms are sustained by an increase in stratification due to the melting ice (Arrigo and Thomas 2004, Vernet et al. 2008). Although sea ice extent is declining in the WAP region (Stammerjohn et al. 2008a), in our study sea ice extent in 2011 (9.37×10^4 km^2) was slightly higher than in 2010 (7.91×10^4 km^2). Sea ice extent is dependent on air temperature, winds, sea surface temperature, currents and stratification,

which in turn are affected by circulation changes related to the El Niño Southern Oscillation (ENSO) and Southern Annular Mode (SAM) (Zwally et al. 2002, Stammerjohn et al. 2008a,b). In 2010, a +SAM coincident with La Niña conditions caused more storms and warm winds from the north, resulting in less sea ice cover and an earlier sea ice retreat. In 2011 there was an early winter switch to –SAM coincident with El Niño conditions, resulting in fewer storms and lower winds, contributing to more sea ice formation and a later sea ice retreat in the spring/summer (S. Stammerjohn, pers. comm.). Thus, chlorophyll-*a* concentrations (and resulting microzooplankton biomass) were higher overall in January 2011, possibly due to higher sea ice cover and later retreat and lower winds, allowing for increased water column stratification and decreased light limitation for phytoplankton.

The southern part of the WAP was more heavily influenced by sea ice, and chlorophyll-*a* and POC concentrations were also generally higher in the south. Elevated chlorophyll-*a* and POC concentrations suggest high food availability for microzooplankton. However, there were no outstanding peaks of ciliate or tintinnid biomass at the station sampled in Marguerite Bay, where chlorophyll-*a* and POC concentrations were extremely high in 2011 (25.1 $\mu g\ L^{-1}$ and 1,239 $\mu g\ L^{-1}$, respectively). This could be due to high predation pressure from larger zooplankton on microzooplankton (Lonsdale et al. 2000). The highest concentration of tintinnids occurred at Station 5 near Charcot Island, which had moderate chlorophyll-*a* and high POC concentrations (Table 2.3). Although microzooplankton biomass was positively correlated with chlorophyll-*a* and POC, changes in these parameters explain only part of the variability in microzooplankton biomass (42% and 64%, respectively). Other bottom-

up and top-down controls affect microzooplankton distributions (i.e., water masses, nutrient concentrations, temperature, salinity, sea ice, zooplankton consumers) (Alder and Boltovskoy 1993, Burkill et al. 1995, Lonsdale et al. 2000, Calbet et al. 2005, Safi et al. 2007, Rose et al. 2009) and consideration of only one variable is likely insufficient to accurately predict microzooplankton distribution.

Overall microzooplankton community structure

Although there were differences in microzooplankton biomass between years, athecate dinoflagellates and ciliates comprised the majority of the microzooplankton biomass in both years, similar to a previous study of the WAP (Calbet et al. 2005) and of the Bellingshausen Sea (Burkill et al. 1995). Aloricate ciliate biomass in our study (0.02 – 20.5 μg C L^{-1}, Table 2.2) was higher than reported by Calbet et al. (2005) (ca. 0 – 10 μg C L^{-1}). Their sampling occurred in December (late austral spring) before the development of large phytoplankton blooms, which could explain the lower ciliate biomass they observed. Overall, microzooplankton abundance and biomass was within the range or slightly higher than in previous studies in the Southern Ocean (Alder and Boltovskoy 1991, Calbet et al. 2005). As the region west of the Antarctic Peninsula is very seasonally productive, supporting large populations of Antarctic krill, marine mammals and birds (Ducklow et al. 2012), it is not surprising that this area has high microzooplankton biomass during the most productive time of year as well.

Overall geographic patterns were not resolved well in the cluster analysis, most likely due to the patchiness of microzooplankton distributions (Garrison 1991a, Umani et al. 1998, Landry et al. 2002). But despite the similarity (>64%) of all sampling stations in

terms of microzooplankton taxonomic composition and biomass, significant latitudinal and cross-shelf trends in microzooplankton distribution occurred.

Latitudinal gradient in microzooplankton biomass

A 'climate gradient' has formed along the WAP, as the warmer, sub-Antarctic climate invades the northern WAP, replacing the typical cold, dry Antarctic climate that still persists in the southern WAP. Therefore, moving south along the climate gradient is akin to moving "back in time" to conditions that persisted in the northern part of the Peninsula in the past (Smith et al. 1999, Ducklow et al. 2012). Microzooplankton may be adjusting to this climate gradient along with other trophic levels. For example, phytoplankton biomass in the north has decreased and phytoplankton assemblages are now dominated by small cells, while phytoplankton biomass in the south has increased and large phytoplankton (i.e., diatoms) dominate (Montes-Hugo et al. 2009). In this study the abundance and biomass of many of the larger microzooplankton taxa (e.g., large tintinnids, large athecate and thecate dinoflagellates) were higher in the south. In 2011 both the thecate dinoflagellate *Protoperidinium antarcticum* and the large tintinnid *Cymatocylis drygalskii* were not found in samples in the northern part of the Peninsula, while abundance and biomass for both species was high in the south (Figure 2.6), paralleling the trend of a shift to relatively large phytoplankton cells in the south (Montes-Hugo et al. 2009). Although there are no long-term studies of WAP microzooplankton, results from our study suggest that microzooplankton may also be adjusting to this climate gradient, possibly adjusting to changes in their food supply.

That aloricate ciliate and tintinnid biomass generally increased with increasing latitude, and biomass maxima generally occurred at the inshore stations most influenced by sea ice at that time of year, is consistent with a previous study that found the highest concentrations of microzooplankton, specifically tintinnids, in the southern, inshore part of the WAP (Bransfield Strait to Bellingshausen Sea, Alder and Boltovskoy 1991). These authors attributed the high tintinnid biomass to their association with sea ice. This result is also consistent with the findings of Nöthig et al. (1991) who reported that the ratio of microzooplankton to phytoplankton biomass was higher in meltwater and concluded that melting ice could be a mechanism to seed pelagic microzooplankton assemblages. Similarly, in our study, the highest ciliate biomass in 2011, which occurred at the southern end of Marguerite Bay (driven by a large bloom of a single ciliate taxa, *Strobilidium* spp.), was associated with the lowest salinity recorded in both years, suggesting a recent influence of sea ice or glacial meltwater. Ciliates similar to forms found in planktonic assemblages are found in sea ice, and can be released to the underlying water column upon ice melt (Garrison 1991b). At this location, melting sea ice could have released ciliates to the water column, influencing the large ciliate bloom that occurred. An alternate explanation for this high ciliate biomass could be an increase in water column stratification with melting sea ice, which stimulates large phytoplankton blooms and could result in high microzooplankton biomass.

Additionally, the latitudinal gradient in microzooplankton bioamss could have been affected by the timing of sample collection. Microzooplankton samples were collected in the northern WAP at the beginning of January and in the southern WAP at the end of January. Microzooplankton assemblages could have changed substantially in

the 20 – 30 days between sample collections in the different regions, complicating interpretation of the results. However, satellite imagery that is ground-truthed with *in situ* sampling shows significant changes in phytoplankton between the north and south (Montes-Hugo et al. 2009). Therefore, although it is a possibility, we do not believe distributions of microzooplankton presented here are an artifact of the timing of sample collection.

Cross-shelf gradient and changes with depth

In addition to latitudinal gradients, we also found differences in microzooplankton taxonomic biomass with distance from shore along the WAP. Although the trends presented here are not as clear as those reported from lower latitudes where microzooplankton biomass significantly decreased with distance from shore (e.g., Southern California Bight; Beers and Stewart 1967, Heinbokel and Beers 1979), in many cases (especially in 2011) microzooplankton biomass was higher closer to shore. Instances where microzooplankton biomass was higher in the midshelf or offshore regions (e.g., aloricate ciliates in 2010, Figure 2.7a), can partially be explained by sea ice dynamics. Productivity along the WAP is not fueled by coastal run-off, as in lower latitudes, but by sea-ice and glacial melt (Leventer 2003, Vernet 2008). Therefore, phytoplankton and microzooplankton biomass can be high along the melting ice edge which is not always in the coastal region, especially further south along the WAP. Additionally, microzooplankton can be associated with different water masses (Burkill et al. 1995, Safi et al. 2007), rather than with distance from shore, which could also explain the cross-shelf variability in microzooplankton biomass.

In both years microzooplankton biomass was uniform in surface waters (10 m and chl-*a* maximum) and was significantly lower at 100 m, consistent with the finding that microzooplankton biomass was positively correlated with chlorophyll-*a* and POC, which were also higher in surface waters. In the Bellingshausen Sea Burkill et al. (1995) also found that microzooplankton concentrations decreased with depth (to 100 m or 125 m). Other studies report subsurface peaks of specific microzooplankton taxa (Boltovskoy and Alder 1992, Calbet et al. 2005), but our sampling resolution was likely not great enough to capture possible subsurface peaks in biomass. Larger protozooplankton (e.g., Radiolaria, Foraminifera) that could have been more abundant at depth than near the surface (Alder and Boltovskoy 1993, Klaas 2001) were not considered in this analysis.

Biological hot spots

Peaks in microzooplankton biomass near Marguerite Bay and the Palmer Deep (i.e., Stations 1 and 3 in Figure 2.5) correspond with previous studies that report these as areas of high primary productivity or high biomass of zooplankton and higher trophic levels (Ashijan et al. 2004, Ribic et al. 2008, Vernet 2008). These are areas along the WAP where deep canyons cut the shallow continental shelf, reaching depths greater than 750 m (Ducklow et al. 2012). Warm, nutrient-rich Upper Circumpolar Deep Water is advected onto the continental shelf via these deep canyons (Martinson et al. 2008). The resulting enhanced productivity in Marguerite Bay made it a focus for the US Southern Ocean Global Ocean Ecosystems Dynamics (US SO GLOBEC) program (Hofmann et al. 2002). Rates of primary productivity are high in Marguerite Bay and phytoplankton assemblages are dominated by diatoms (Clarke et al. 2008, Vernet et al. 2008). High

abundance and biomass of large krill (*Euphausia superba*) are found in Marguerite Bay due to the high quality food, but possibly also due to the persistent sea ice cover every winter (Perovich et al. 2004, Hyatt et al. 2011), which is essential for larval krill survival and recruitment (Daly 1990, Quetin et al. 1996), and small gyres in Marguerite Bay that may retain krill populations (Ashjian et al. 2004, Beardsley et al. 2004). This productivity fuels populations of higher trophic levels such as penguins and marine mammals (Ribic et al. 2008, Erdmann et al. 2011, Friedlaender et al. 2011) and high primary production may also lead to high microzooplankton biomass here as well.

SUMMARY AND CONCLUSION

This study is the first comprehensive description of microzooplankton community structure along the WAP. Microzooplankton assemblages were dominated by aloricate ciliates and athecate dinoflagellates and showed distinct between-year variability. Microzooplankton biomass was higher in 2011 compared to 2010, likely driven by sea ice and phytoplankton bloom dynamics. There were distinct latitudinal, cross-shelf, and depth gradients in microzooplankton biomass. In general, microzooplankton biomass was higher in the south compared to the north, in surface waters compared to depth, and to a lesser extent, near shore vs. offshore. Sea ice dynamics and circulation patterns most likely affected microzooplankton biomass distributions with distance from shore. Microzooplankton biomass was positively correlated with chlorophyll-*a* and POC, but other parameters also affect microzooplankton distribution, such as meso- and macrozooplankton consumers, temperature, and salinity. Marguerite Bay, historically a biological hot spot (Ashjian et al. 2004, Vernet et al. 2008, Erdmann et al. 2011, Friedlaender et al. 2011), is a center of high microzooplankton biomass as well. Removing samples collected in Marguerite Bay from the analysis of microzooplankton biomass between the north and south generally did not affect the trend of higher biomass in the south.

The WAP is experiencing rapid warming with a decrease in sea ice, and like other components of the WAP food web, microzooplankton may be adjusting their distribution to reflect these changes in their environment. This study provides evidence for how the environment affects microzooplankton distribution, as well as a reference point for

subsequent studies to assess how future changes will affect microzooplankton community structure and the microbial food web in the WAP.

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Table 2.1. Microzooplankton cell volume and carbon content ± S.E used to calculate biomass of major taxa.

	Volume ($\times 10^3$ µm^3)	Carbon ($\times 10^3$ pg C)
Ciliates		
Strombidium spp. (<30 µm)	2.67 ± 0.121	0.507 ± 0.023
Strombidium spp. (>30 µm)	20.7 ± 1.01	3.94 ± 0.192
Strombidium conicum	7.61 ± 0.273	1.45 ± 0.052
Tontonia spp.	3.01 ± 0.178	0.572 ± 0.034
Laboea spp.	5.35 ± 0.710	1.02 ± 0.135
Strobilidium spp. (<30 µm)	0.974 ± 0.610	0.185 ± 0.012
Strobilidium spp. (>30 µm)	68.0 ± 4.88	12.9 ± 0.927
Didinium sp.	140 ± 12.7	26.6 ± 2.42
Myrionecta rubra	1.70 ± 0.068	0.323 ± 0.013
Euplotes sp.	225 ± 11.4	42.8 ± 2.16
unidentified ciliate *a*	28.2 ± 1.68	5.35 ± 0.320
Other	1,320 ± 95	0.251 ± 0.018
Tintinnids		
Salpingella spp.	1.69 ± 0.065	0.534 ± 0.003
Laackmaniella spp.	212 ± 6.58	11.7 ± 0.349
Cymatocylis spp.	177 ± 10.8	9.83 ± 0.574
Cymatocylis drygalskii	508 ± 7.02	27.4 ± 0.372
Codonellopsis gaussi	58.9 ± 1.53	3.56 ± 0.081
Codonellopsis brasiliensis	49.5 ± 1.36	3.07 ± 0.072
Epiplocyloides antarctica	116 ± 6.79	6.64 ± 0.360
Athecate dinoflagellates		
Gyrodinium sp.	131 ± 13.2	11.4 ± 0.917
Small (<60 µm)	27.7 ± 2.53	3.20 ± 0.242
Large (>60 µm)	175 ± 23.9	14.2 ± 1.52
Thecate dinoflagellates		
Protoperidinium spp.	11.7 ± 1.48	1.56 ± 0.156
Protoperidinium antarcticum	327 ± 16.0	24.8 ± 1.00
Small (<30 µm)	1.58 ± 0.081	0.313 ± 0.013
Medium (30 – 60 µm)	24.7 ± 1.89	2.95 ± 0.182
Other		
Dictyocha speculum (silicoflagellate)	5.23 ± 0.301	0.418 ± 0.024
unidentified flagellate	0.864 ± 0.030	0.192 ± 0.005

Table 2.2. Microzooplankton abundance (ind. L^{-1}) and biomass (µg C L^{-1}) averages and ranges (in parentheses) at three different depths and 0-100 m integrated abundance ($\times 10^6$ ind. m^{-2}) and biomass (mg C m^{-2}) average and range (in parentheses). Data from stations sampled in the Palmer Antarctica Long-Term Ecological Research study area in 2010 and 2011.

	2010		2011	
	Abundance	**Biomass**	**Abundance**	**Biomass**
Aloricate Ciliates				
10 m	2,002 (460 – 3,360)	3.8 (0.2 – 12.0)	4,423 (660 – 39,963)	6.0 (0.4 – 21.5)
Chl-*a* maximum	1,633 (350 – 5,820)	3.5 (0.2 – 15.1)	3,029 (410 – 30,363)	4.5 (0.1 – 20.5)
100 m	345 (80 – 780)	0.6 (0.02 – 1.6)	405 (150 – 798)	0.5 (0.08 – 1.8)
Integrated (0-100m)	129 (30 – 373)	264 (16 – 915)	224 (0.003 – 1,998)	333 (28 – 1,278)
Tintinnids				
10 m	558 (0 – 4,170)	0.5 (0 –3.7)	637 (60 – 2,785)	2.1 (0.03 – 9.2)
Chl-*a* maximum	412 (0 – 3,550)	0.6 (0 – 4.9)	617 (60 – 1,750)	1.4 (0.06 – 6.3)
100 m	52 (0 – 510)	0.2 (0 – 2.2)	162 (11 – 1,144)	0.5 (0.006 – 1.3)
Integrated (0-100m)	32 (0 – 271)	44 (0 – 370)	43 (0.3 – 124)	117 (10 – 410)
Athecate dinoflagellates				
10 m	1,000 (176 – 2,542)	7.0 (1.0 – 16.7)	2,972 (565 – 9,775)	19.8 (2.7 – 61.1)
Chl-*a* maximum	987 (272 – 2,730)	7.1 (1.9 – 24.1)	2,096 (323 – 9,120)	12.0 (1.9 – 53.0)
100 m	89 (30 – 170)	0.4 (0.1 – 1.0)	333 (39 – 985)	1.6 (0.2 – 4.7)
Integrated (0-100m)	72 (17 – 170)	510 (108 – 1,489)	159 (0.2 – 653)	964 (262 – 4,049)
Thecate dinoflagellates				
10 m	853 (200 – 2,431)	0.4 (0.08 – 1.3)	2,439 (490 – 7,370)	3.0 (0.2 – 11.9)
Chl-*a* maximum	714 (60 – 3,675)	0.3 (0.06 – 1.2)	1,564 (440 – 6,688)	2.0 (0.3 – 11.2)
100 m	121 (20 – 590)	0.1 (0.01 – 0.6)	243 (83 – 633)	0.4 (0.05 – 1.4)
Integrated (0-100m)	55 (14 – 226)	28 (8 – 76)	126 (0 – 405)	165 (32 – 709)
Silicoflagellate				
10 m	856 (0 – 10,690)	0.34(0 – 4.5)	682 (40 – 5,900)	0.3 (0.02 – 2.5)
Chl-*a* maximum	615 (0 – 5,060)	0.3 (0 – 2.1)	343 (0 – 2,030)	0.1 (0 – 0.8)
100 m	69 (0 – 750)	0.03 (0 – 0.3)	25 (0 – 270)	0.01 (0 – 0.1)
Integrated (0-100m)	50 (0 – 474)	21 (0 – 198)	31 (0.0007 – 191)	13 (0.2 – 80)
unidentified flagellate				
10 m	26,388 (50 – 108,500)	5.1 (0.01 – 20.8)	602 (0 – 6,640)	0.1 (0 – 1.3)
Chl-*a* maximum	25,217 (0 – 104,200)	4.8 (0 – 20.0)	189 (0 – 1,680)	0.04 (0 – 0.3)
100 m	455 (0 – 7,540)	0.09 (0 – 1.5)	17 (0 – 80)	0.003 (0 – 0.02)
Integrated (0-100m)	1,723 (0.8 – 5,916)	331 (0.1 – 1,136)	20 (0.0004 – 135)	4 (0.02 – 26)

Table 2.3. Chlorophyll-*a* ($\mu g\ L^{-1}$) and particulate organic carbon (POC, $\mu g\ L^{-1}$) values at three different depths, and integrated chlorophyll-*a* ($\times 10^3\ \mu g\ m^{-2}$) and POC ($\times 10^6\ \mu g\ m^{-2}$) at select stations in the Palmer Antarctica Long-Term Ecological Research study area in 2010 and 2011. Only stations of interest referred to in the text (see Figure 2.1) are shown. Data courtesy of O. Schofield and H. Ducklow and can be found at http://pal.lternet.edu/data/. (–) indicates data not available.

	2010		**2011**	
	Chl-*a*	**POC**	**Chl-*a***	**POC**
Station 1 (64.9°S, 64.4°W)				
10 m	1.13	224	1.62	164
Chl-*a* max (20 m)	1.14	220	0.540	69
100 m	0.066	29	0.063	22
Integrated (0-100 m)	70.8	14.4	51.2	6.4
Station 2 (65.1°S, 67.1°W)				
10 m	-	-	1.01	134
Chl-*a* max (40 m)	-	-	1.08	117
100 m	-	-	0.414	50
Integrated (0-100 m)	-	-	86.5	10.1
Station 3 (68.6°S, 71.0°W)				
10 m	-	-	6.72	893
Chl-*a* max (20 m)	-	-	8.64	723
100 m	-	-	0.427	21
Integrated (0-100 m)	-	-	507	46.8
Station 4 (69.0°S, 75.6°W)				
10 m	2.98	465	-	-
Chl-*a* max (35 m)	4.22	439	-	-
100 m	1.83	51	-	-
Integrated (0-100 m)	315	31.9	-	-
Station 5 (69.5°S, 75.5°W)				
10 m	8.51	598	4.29	895
Chl-*a* max (30 m)	1.16	97	13.3	892
100 m	0.121	33	-	-
Integrated (0-100 m)	234	18.0	-	-

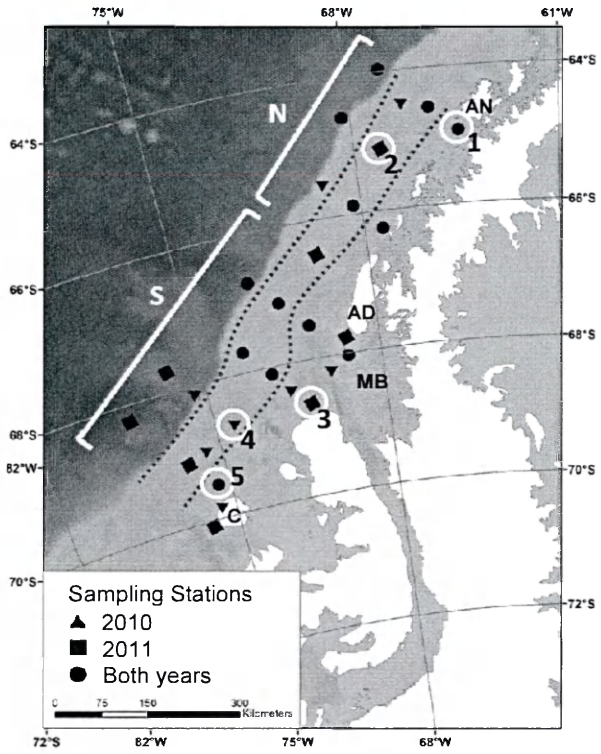
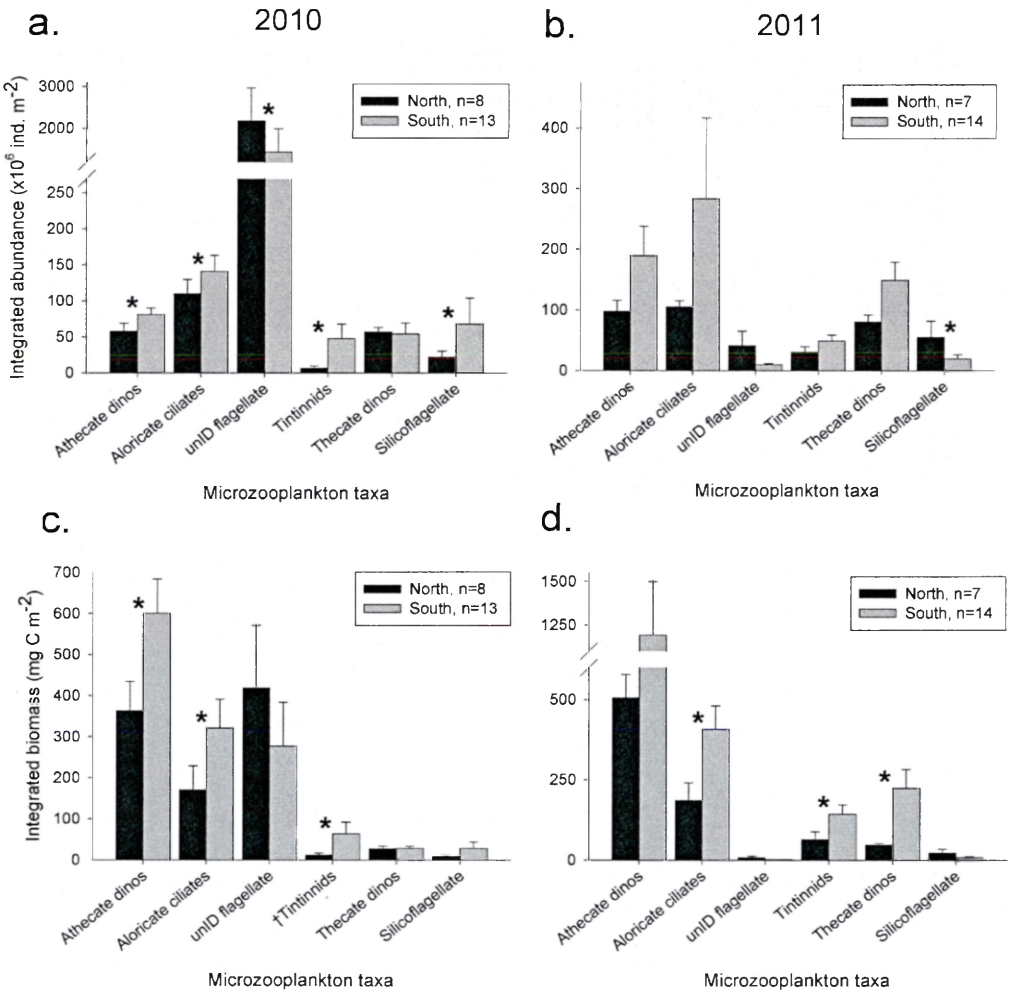


Figure 2.1. Map of the Palmer Antarctica Long-Term Ecological Research study region where whole water samples were collected for microzooplankton community composition in 2010 and 2011. AN: Anvers Island, AD: Adelaide Island, MB: Marguerite Bay, C: Charcot Island. Palmer Station is located on Anvers Island. The Palmer Deep canyon is located just south of Anvers Island, and the Marguerite Trough is located just south of Adelaide Island. Northern and southern sub-regions are indicated. Inshore, midshelf, and offshore regions are separated by dashed lines. All region divisions are based on hydrographic and sea-ice conditions (Martinson et al. 2008, Stammerjohn et al. 2008a). Select stations of interest referred to in the text are circled and numbered. Light gray indicates the continental shelf, which is about 200 km wide and averages 430 m in depth. The light/dark gray interface indicates the shelf break to waters ~3000 m deep (Ducklow et al. 2012).

Figure 2.2. Integrated abundance (0-100m) in (a) 2010 and (b) 2011, and integrated biomass (0-100m) in (c) 2010 and (d) 2011 for the major microzooplankton taxa in the northern and southern sub-regions of the Western Antarctic Peninsula. Error bars are standard errors, asterisks (*) indicates statistically significant ($p<0.05$) differences in abundance and biomass between sub-regions (north vs. south). † indicates a significant interaction between sub-region (north, south) and location (inshore, midshelf, offshore) in the 2-way ANOVA for integrated tintinnid biomass in 2010. For statistical analysis, data were transformed using the natural log transformation to fit the normality and homogeneity of variance assumptions of the ANOVA. See Figure 2.1 for map showing division of northern and southern sub-regions.


a.
2010
b.
2011
Integrated abundance (x10^6 ind. m^-2)
North, n=8
South, n=13
North, n=7
South, n=14
Athecate dinos
Aloricate ciliates
unID flagellate
Tintinnids
Thecate dinos
Silicoflagellate
Microzooplankton taxa
c.
d.
Integrated biomass (mg C m^-2)
†Tintinnids
Microzooplankton taxa

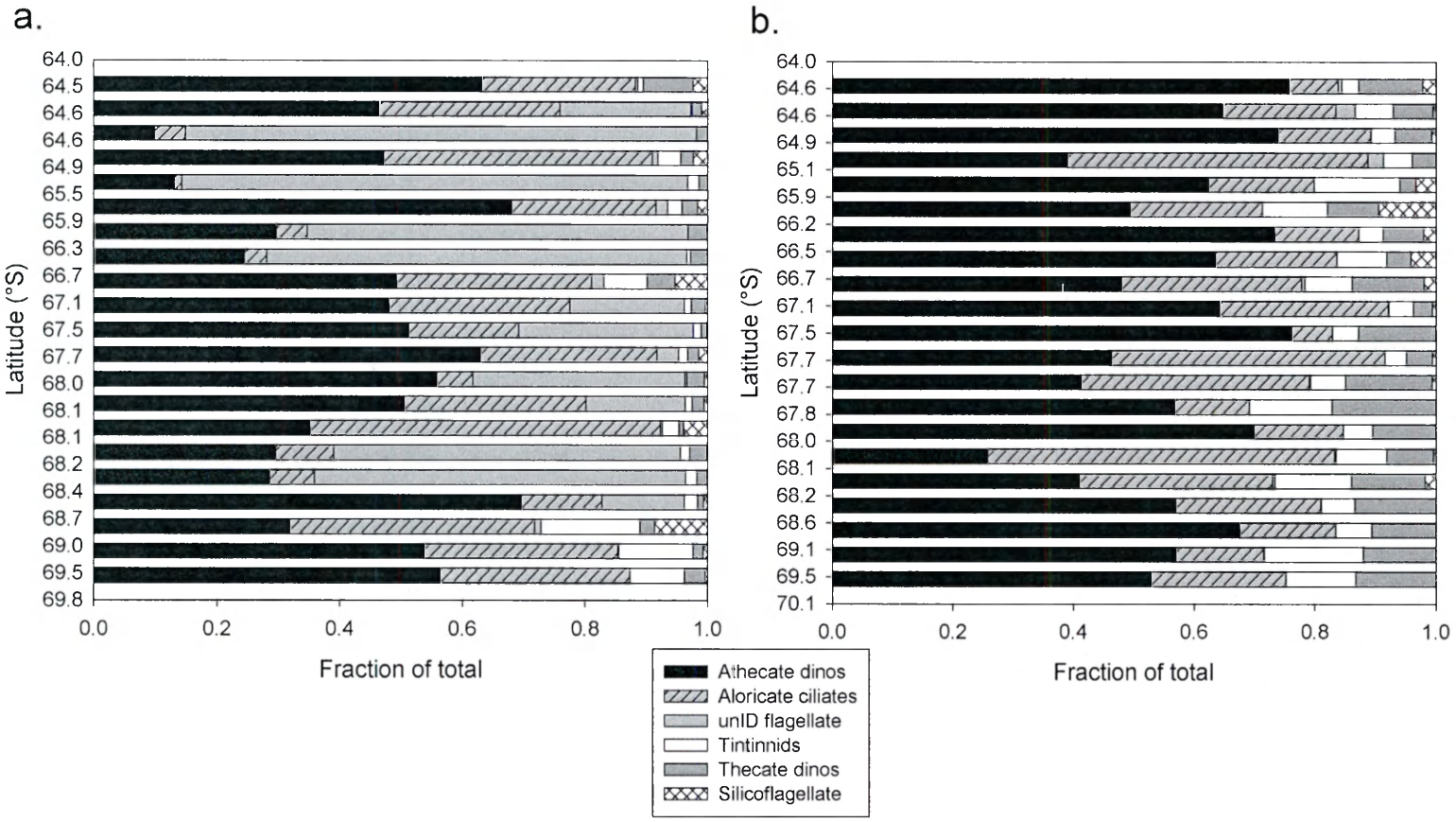


Figure 2.3. The % contribution of each microzooplankton taxa to total integrated (0-100m) microzooplankton biomass in (a) 2010 and (b) 2011.

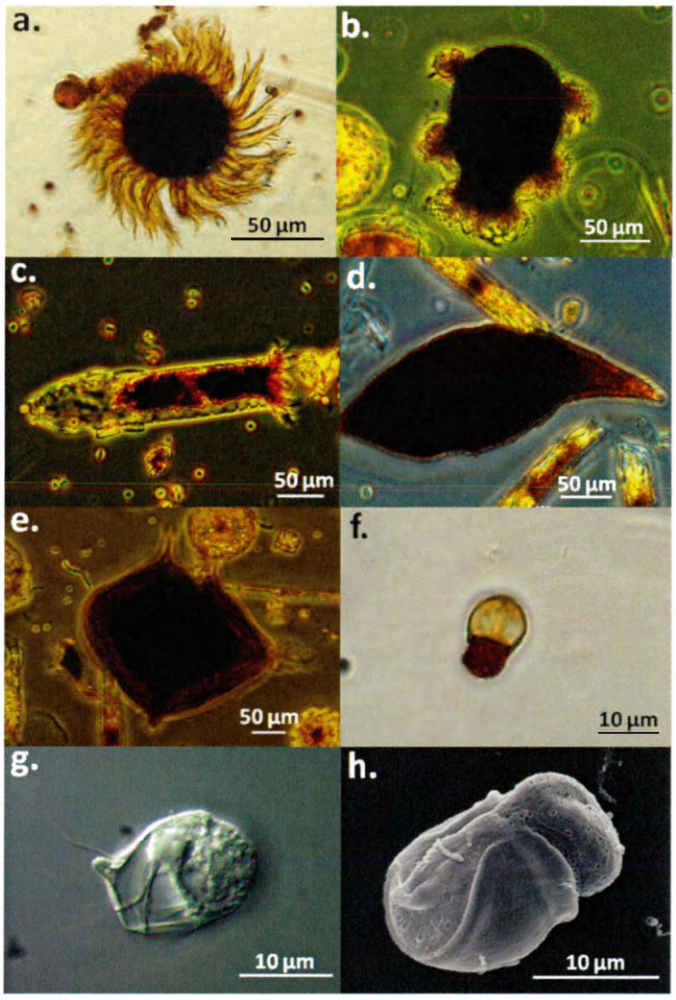


Figure 2.4. Micrographs of conspicuous microzooplankton taxa. Aloricate ciliates (a) *Strobilidium* sp. and (b) *Didinium* sp., (c) tintinnid *Laackmaniella* sp., (d) large athecate dinoflagellate *Gyrodinium* sp., (e) large thecate dinoflagellate *Protoperidinium antarcticum*, and (f – h) and unidentified flagellate. Samples in micrographs (a – f, h) preserved in 6-8% acid Lugol's, and (g) preserved in 1% glutaraldehyde. Photos taken with (a – f) phase contrast light microscopy, (g) differential interference contrast (DIC) microscopy, and (h) scanning electron microscopy (SEM). Photo (g) by D. Wayne Coats, photo (h) by Patrice Mason.

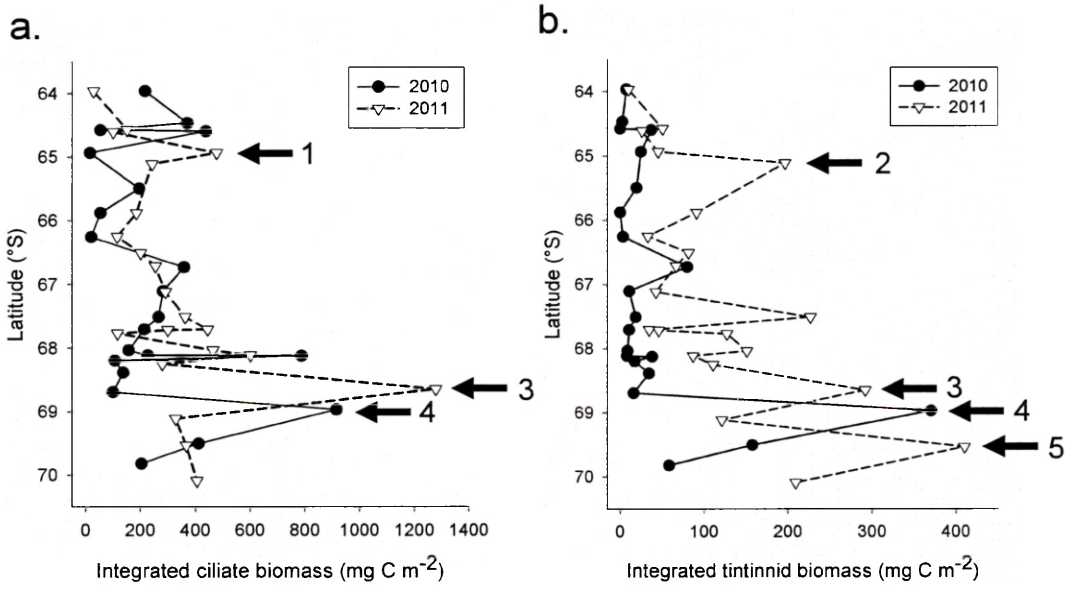


Figure 2.5. Latitudinal gradient in integrated biomass (0-100m) of (a) aloricate ciliates and (b) tintinnids. Each point represents n=1. Select stations of interest referred to in text are labeled; see Figure 2.1 for station locations.

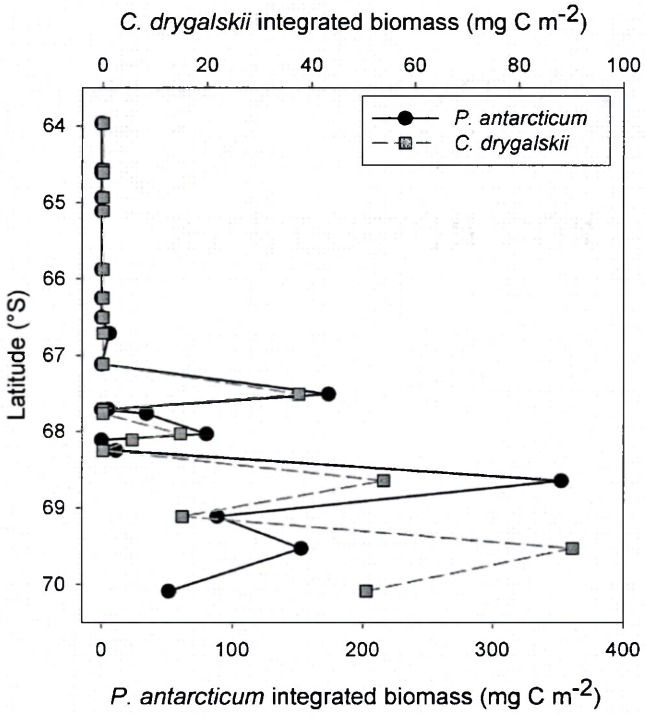


Figure 2.6. Latitudinal gradient in integrated biomass (0-100m) of the large thecate dinoflagellate *Protoperidinium antarcticum* and the large tintinnid *Cymatocylis drygalskii* in 2011. Each point represents n=1.

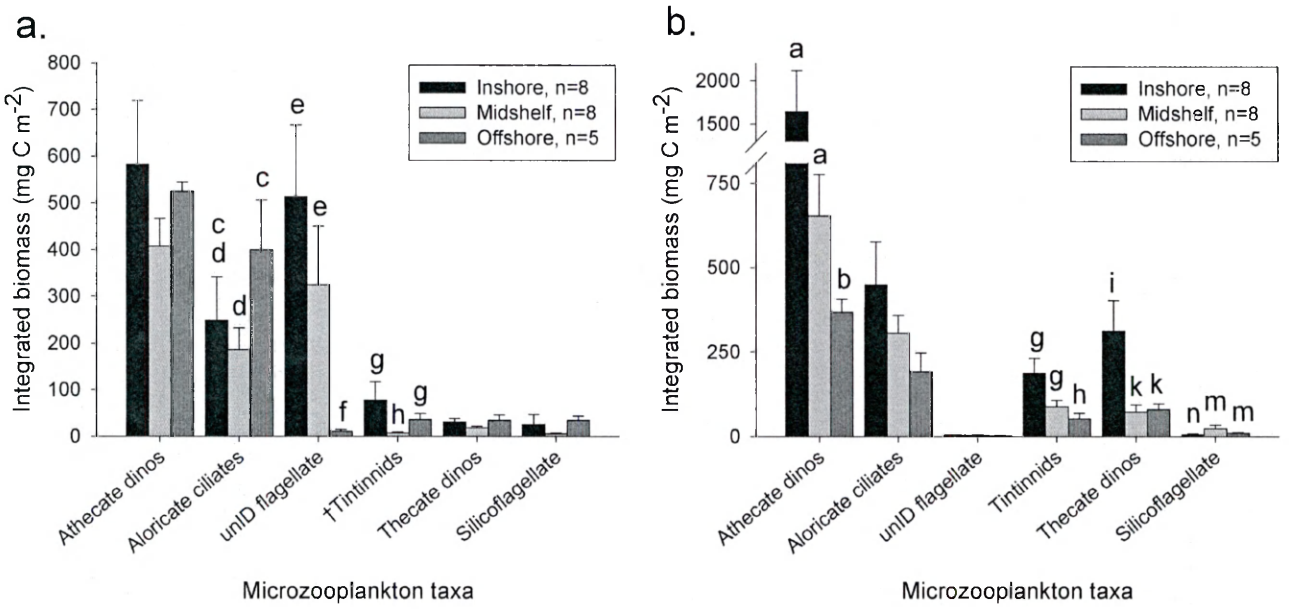


Figure 2.7. Integrated biomass (0-100m) for the major microzooplankton taxa across the shelf gradient in (a) 2010 and (b) 2011. The y-axis in (a) equals the y-axis in (b) before the axis break. Error bars are standard errors, labels indicate statistically significant ($p<0.05$) differences in biomass among locations: a > b, c > d, e > f, g > h, i > k, m > n. † indicates significant interactions between sub-region (north, south) and location (inshore, midshelf, offshore) in the 2-way ANOVA for integrated tintinnid biomass in 2010. For statistical analysis, data were transformed using the natural log transformation to fit the normality and homogeneity of variance assumptions of the ANOVA. See Figure 2.1 for map showing division of cross-shelf sub-regions.

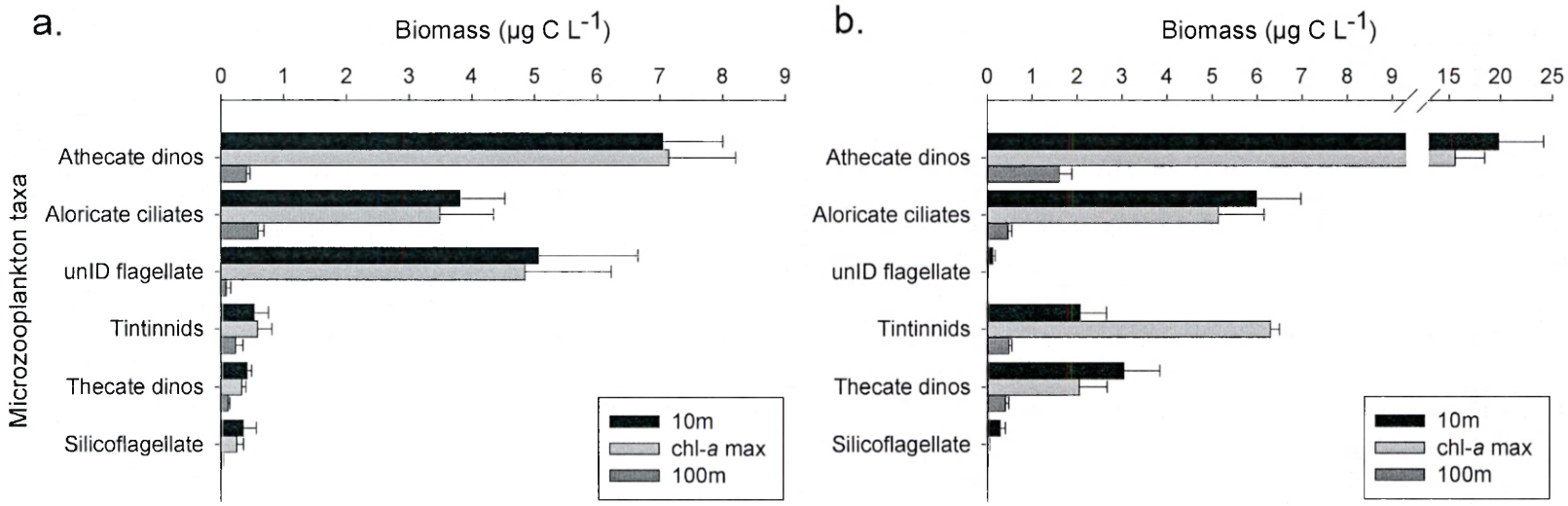


Figure 2.8. Biomass of the major microzooplankton taxa at different depths in (a) 2010 and (b) 2011. Error bars are standard errors, n=21 for each depth. For all taxa, biomass at 10m and the chl-*a* max were not statistically different while the biomass at 100m was significantly lower than at shallower depths ($p<0.05$). The exception to the latter was the tintinnids in 2010 for which there was no significant difference in biomass with depth. For statistical analysis, data were transformed using the natural log transformation to fit the normality and homogeneity of variance assumptions of the ANOVA.

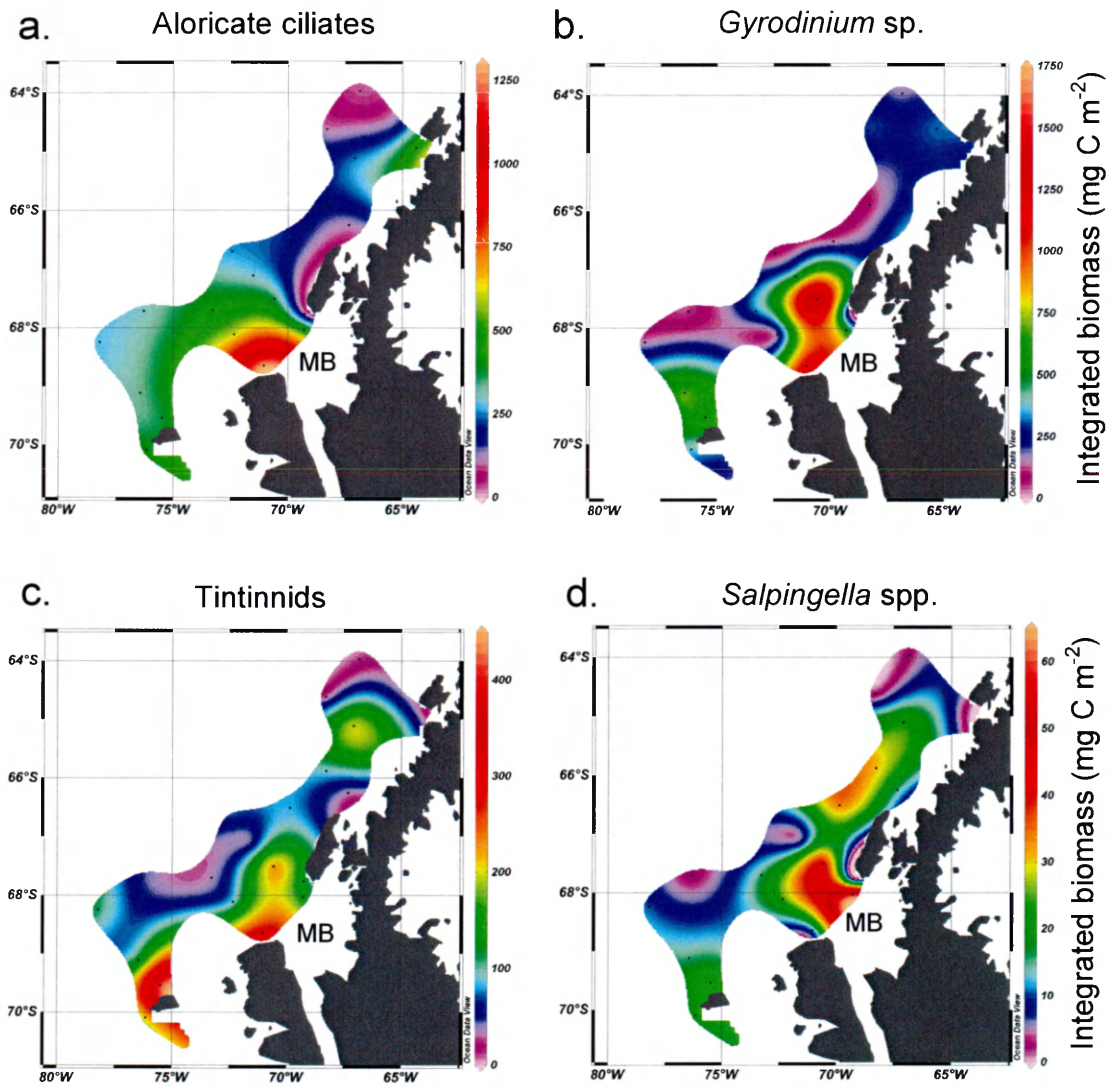


Figure 2.9. Integrated biomass (0-100m) in 2011 for four microzooplankton taxa, (a) total ciliates, (b) dinoflagellate *Gyrodinium* sp., (c) total tintinnids, and (d) tintinnids *Salpingella* spp. MB: Marguerite Bay.

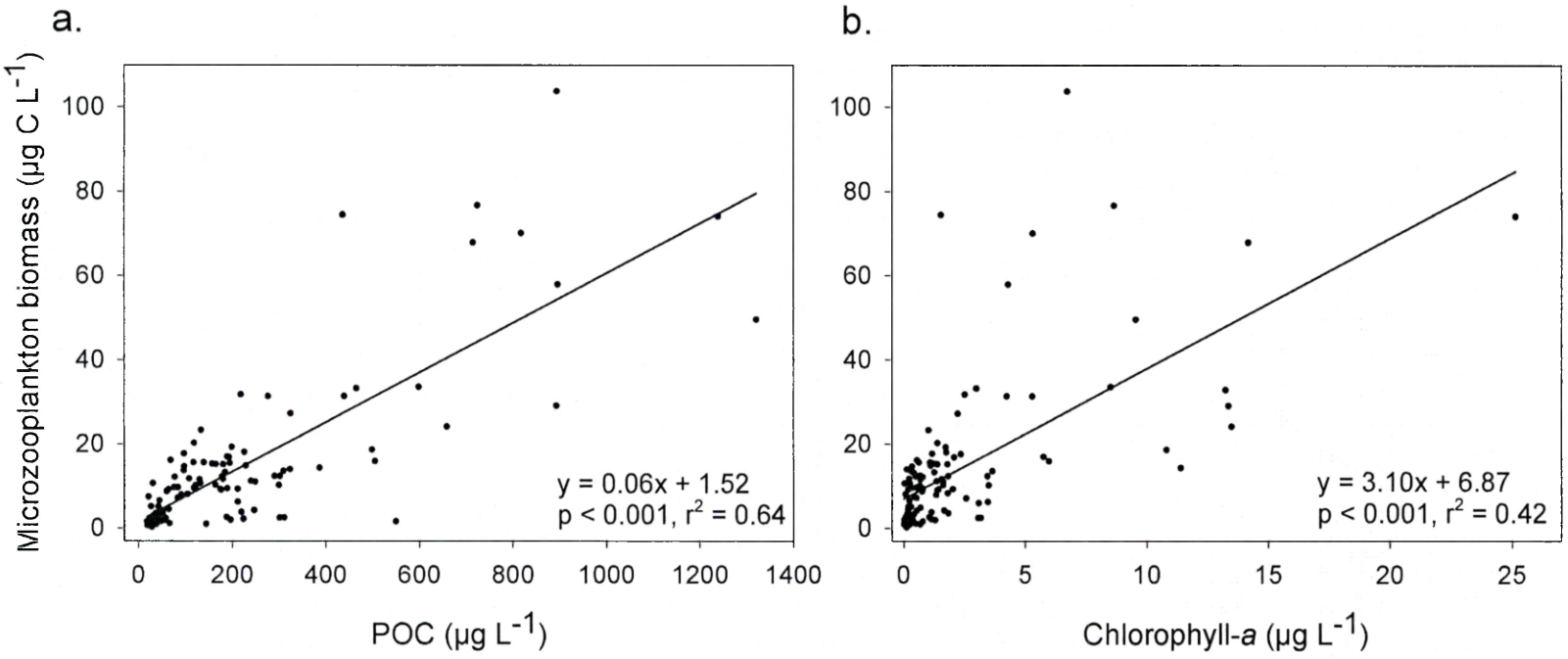


Figure 2.10. Relationship between (a) particulate organic carbon (POC), and (b) chlorophyll-*a* vs. total microzooplankton biomass. Each point represents data from a specific station and depth where microzooplankton biomass was determined and where (a) POC (data courtesy of H. Ducklow), or (b) chlorophyll-*a* (data courtesy of O. Schofield) values were available. Includes data from 2010 and 2011, as well as all three depths sampled (10m, chl-*a* maximum and 100m).

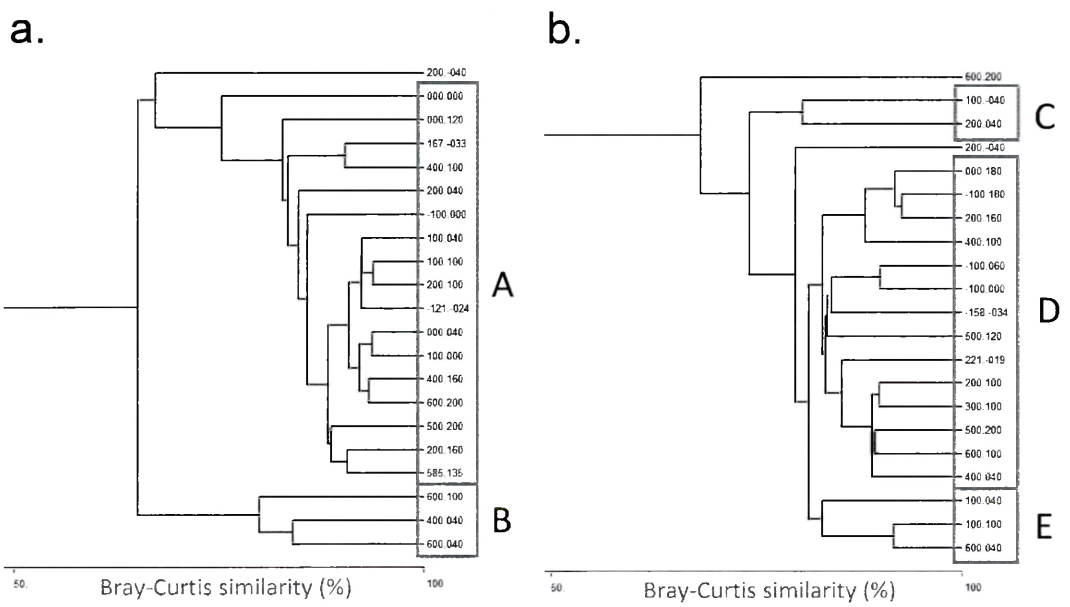


Figure 2.11. Dendrograms of cluster analyses based on Bray-Curtis similarity among stations along the Western Anatarctic Peninsula in (a) 2010 and (b) 2011. The station numbers are demarcations used by the Palmer Antarctica Long-Term Ecological Research (LTER) project to denote station locations along the LTER sampling grid. First three numbers indicate orientation along the north-south axis of WAP, from line 600 (furthest north) to line -100 (furthest south). These lines are oriented perpendicular to the coast and are spaced 100 km apart along the Peninsula. Second three numbers indicate distance from shore (in km) from an arbitrary zero line defining the coastline. Higher numbers indicate stations further from shore, negative numbers indicate stations in bays/inlets. For a more complete description of station labels and coordinates, see Waters and Smith (1992).

CHAPTER 3

Microzooplankton grazing along the Western Antarctic Peninsula

ABSTRACT

The significance of microzooplankton as grazers in pelagic ecosystems has been established, yet relatively few studies of microzooplankton grazing, compared to that of macrozooplankton, have been conducted in the Southern Ocean. We report phytoplankton and bacterial growth and grazing mortality rates along the Western Antarctic Peninsula (WAP), a region of rapid climate change, as part of the Palmer Antarctica Long-Term Ecological Research project. Growth and grazing rates were determined by dilution experiments at select stations along the WAP in January 2009 – 2011 and in the nearshore waters near Palmer Station in February – March 2011. Microzooplankton exerted higher grazing pressure on bacteria compared to phytoplankton along the WAP, and also exhibited selective grazing on smaller phytoplankton (picoautotrophs and nanophytoplankton) and on the more actively growing (high nucleic acid) bacterial cells. Among all phytoplankton size classes, growth rates ranged from undetectable (NS) – 0.99 d^{-1}, grazing mortality rates were NS – 0.56 d^{-1}, and microzooplankton removed <100% of phytoplankton production in all but one experiment. For high and low nucleic acid content bacteria, growth rates were NS – 0.95 d^{-1} and grazing mortality rates were NS – 0.43 d^{-1}; microzooplankton often removed >100% of bacterial production. A strong wind storm at Palmer Station likely altered microbial food web dynamics by reducing the microzooplankton grazing impact on phytoplankton while increasing the grazing impact on high nucleic acid content bacteria. There was a significant (albeit weak) exponential relationship between temperature vs. phytoplankton mortality, although the range of experimental temperatures was very small. This study provides a reference point of microzooplankton grazing impact along

the WAP in the summer and contributes valuable information to studies modeling the flow of carbon through the WAP food web, improving our ability to predict climate-induced changes in the WAP ecosystem.

INTRODUCTION

Microzooplankton are significant grazers on phytoplankton and bacteria, can consume a wide range of prey types and sizes (Sherr and Sherr 2002), and influence phytoplankton and bacterial assemblages due to selective feeding (Stoecker 1988, Banse 1992, Sherr and Sherr 1994). Although the importance of microzooplankton grazers in the Southern Ocean has been established (von Brockel 1981, Buck and Garrison 1983, Hewes et al. 1985, Heinbokel and Coats 1986), there have been relatively few studies of microzooplankton, compared to meso- and macrozooplankton (i.e., >200 μm). This is due in part to the historical focus on the role of krill as grazers, along with the inherent difficulty of studying microzooplankton and measuring their grazing rates (Landry and Hassett 1982, Dolan et al. 2000, Calbet and Landry 2004, Dolan and McKeon 2004), especially in an extreme environment.

Studies that quantify microzooplankton grazing impact on primary producers in the Southern Ocean report extremely variable phytoplankton mortality rates (Garrison 1991), from low-to-undetectable grazing (maximum grazing mortality = 0.26 d^{-1}, Caron et al. 2000) to removal of >100% of primary production (maximum grazing mortality = 2.36 d^{-1}, Pearce et al. 2010). Pearce et al. (2008) reported that micrograzers removed up to 762% of primary production (grazing mortality = 1.13 d^{-1}) in the marginal ice zone near Davis Station, East Antarctica, and concluded that microzooplankton are key to controlling and ending phytoplankton blooms at the end of summer in coastal Antarctic waters. On the other hand, Caron et al. (2000) assessed microzooplankton grazing in the Ross Sea and found grazing rates statistically higher than zero in only nine of 34 experiments. Of those nine, all rates were low (<0.26 d^{-1}), and they concluded that much

of the phytoplankton bloom was not grazed but removed by aggregation and sinking. In the Ross Sea, these low to negligible grazing rates may be due to very low water temperatures that constrain microzooplankton activity, and the presence of large colonies of *Phaeocystis antarctica* and possibly large, unpalatable diatoms that potentially deter microzooplankton grazing (Caron et al. 2000).

In a meta-analysis of the role of temperature on growth rates of aquatic protists, Rose and Caron (2007) proposed that temperature differentially affects heterotrophic protist and phytoplankton growth rates, which could lead to imbalances between phytoplankton growth and mortality in this system. Colder temperatures constrain the growth of heterotrophic protists to a higher degree than phytoplankton, potentially causing low microzooplankton grazing rates at very low temperatures (as seen in Caron et al. 2000). This release from microzooplankton grazing pressure could allow for the large phytoplankton blooms often observed in the Southern Ocean (Rose and Caron 2007).

In addition to their importance as herbivores, microzooplankton are key consumers of bacterioplankton. Flagellate populations can graze from 25% to over 100% of the measured daily production of bacterioplankton (Sherr and Sherr 1994) and can considerably alter bacterial assemblages by selective feeding (Sherr et al. 1992, Sherr and Sherr 1994). Bacteria in coastal Antarctic waters ultimately depend on phytoplankton production for organic matter, and therefore should be coupled to the phytoplankton dynamics. In a recent time series analysis (2003 – 2011) of bacterial production along the Western Antarctic Peninsula (WAP) during austral summer, bacterial production was positively correlated with phytoplankton biomass (Ducklow et al. 2012b). However, Bird

and Karl (1999) reported that bacteria were not correlated with chlorophyll-*a* during a spring bloom in the northern Antarctic Peninsula and concluded that the bacterial response to the phytoplankton bloom was likely suppressed by grazing by heterotrophic nanoflagellates. Summer bacterial abundances in the WAP are relatively constant (Ducklow et al. 2012a), which could be explained by constant microzooplankton grazing pressure.

The WAP is a region undergoing rapid warming, with 1°C increase in average winter air temperature each decade since 1950 (Smith et al. 1996, Vaughan et al. 2003, Ducklow et al. 2012a). The waters along the WAP are seasonally productive and support large populations of zooplankton (e.g., krill) and top predators such as penguins, seals, and whales (Ducklow et al. 2007, Ross et al. 2008, Vernet et al. 2008). Many components of the food web in this region have been studied extensively (Ducklow et al. 2012a), while microzooplankton have been largely overlooked. A previous study that assessed microzooplankton grazing rates near our sampling area (to the north, near the tip of the WAP) shows that although there was significant variability in phytoplankton growth (0 – 1.16 day^{-1}) and mortality (0 – 0.29 day^{-1}), a balance between phytoplankton growth and mortality was observed in half of the experiments (Tsuda and Kawaguchi 1997).

In this study we report the first comprehensive analysis of microzooplankton grazing rates along the WAP. We present phytoplankton and bacterial growth and mortality rates as measured by the dilution method (Landry and Hassett 1982) at select locations along the WAP, as well as in the nearshore waters near Palmer Station. We investigate selective feeding by microzooplankton on different phytoplankton size classes and bacterial types, in addition to temperature effects on microzooplankton grazing rates.

These measurements of microzooplankton grazing rates on phytoplankton and bacteria will help us better understand microbial food web dynamics in a region of rapid climate change, and will provide a reference point for future studies.

MATERIALS AND METHODS

Study site

As part of the Palmer Antarctica Long-Term Ecological Research (PAL LTER) project (Ducklow et al. 2012a), phytoplankton and bacterial growth and mortality rates were calculated using the dilution method (Landry and Hassett 1982) on research cruises aboard the R/V *Laurence M. Gould* in January (austral summer) 2009, 2010 and 2011 (a total of 12 experiments) in continental shelf waters, along the WAP. Experiments were also conducted in the near-shore waters near Palmer Station, Antarctica (sampling location: 64.78°S, 64.04°W) in February through March 2011 (seven experiments) (Figure 3.1).

Experimental set-up

On the annual January research cruises, water samples for each experiment were collected within the mixed layer (10 m – 50 m) using 12 L Niskin bottles mounted on a CTD rosette. At Palmer Station water samples were collected at a depth of 5 or 10 m using 5 L Go-Flo bottles. All experimental bottles, silicone tubing, and other materials were acid-washed (10% HCl) and rinsed with Milli-Q water prior to use and between experiments. Plastic Nitrile gloves were worn throughout all sampling and experimental manipulations. Filtered seawater (FSW) for experiments was generated by gentle gravity filtration using cartridge filters (0.2 μm pore size) and "whole" seawater was collected by gentle, reverse-flow filtration through 200 μm mesh to exclude mesozooplankton. According to Caron and Dennett (1999), gentle gravity filtration using cartridge filters does not cause detectable changes in dissolved organic carbon or inorganic nutrients.

A dilution series of 20, 40, 60, 80, and 100% whole seawater was prepared in 9 L clear polycarbonate carboys by first adding the appropriate volume of FSW to each carboy, and then gently siphoning whole water directly from the Niskin bottles into the carboys (with outflow carefully maintained under the FSW level to avoid cell breakage). Macronutrients were not added to the experimental carboys on the LTER cruises because phytoplankton growth in the Southern Ocean is generally not limited by macronutrients (silicate, nitrate, and phosphate), which occur at high concentrations (Hayes et al. 1984, Martin 1990). All treatments on the 2011 research cruise were enriched with $FeCl_3$ to a final iron concentration of 5 nM to avoid iron limitation. A control without iron enrichment was also prepared. Subsequent studies indicate iron limitation only at the offshore stations along the WAP (R. Sherrell and M. Séguret, pers. comm.); therefore, iron additions in our experiments, which were conducted at more coastal stations with no evidence of iron limitation, were likely unnecessary but would not have altered the results.

In two experiments at Palmer Station (15-Feb and 16-Feb), carboys were enriched with inorganic nutrients and trace metals (with final concentrations of 10 μM nitrogen as NH_4Cl, 1 μM phosphorus as Na_2HPO_4, 5 nM iron as $FeCl_2$, and 0.1 nM magnesium as $MgSO_4$) to assess the effects of nutrient addition on the dilution experiments. An additional 100% treatment carboy was prepared without nutrient additions as a control. Experiments where the difference between the 100% treatment and Fe/macronutrient control were significantly different (Student's t-test, $p < 0.05$) were noted, but data were not corrected.

Water from each carboy/dilution treatments was apportioned into triplicate 1.2 L clear polycarbonate bottles. The experimental bottles were placed in an outside incubator with running seawater to maintain ambient temperatures. Two layers of gray window screening were used to reduce light to 20-25% of surface irradiance; the bottles were incubated for 72 hours (Caron et al. 2000).

Sample analyses

Fluorometric chlorophyll-a analysis

Seawater samples (200-1000 ml) were taken at initial and final time points (from the carboys, and incubation bottles, respectively) and filtered in the dark onto 25-mm Whatman GF/F glass fiber filters (nominal pore size 0.7 µm, effective pore size is smaller, Chavez et al. 1995) using a vacuum of <200 mm Hg. Filtrate from the 100% treatments were analyzed for initial and final nutrient concentrations. Filters were frozen at -80°C and stored until analysis at Palmer Station. After removing filters from the freezer, the pigments were extracted in 90% acetone for 24 hours in the dark at -20°C and chlorophyll-*a* concentrations were determined fluorometrically using a Turner Designs 10AU digital fluorometer.

Bacteria and phytoplankton enumeration

Autofluorescent and heterotrophic bacteria and autofluorescent particles (phytoplankton, <40µm) were counted live immediately after initial or final samples were taken using a BD Accuri® C6 flow cytometer equipped with a blue laser beam (50 mW, 488 nm) and CFlow Plus Software. All gains were set in logarithmic mode with a seven-

decade channel scale to avoid modifying channel voltages. Fluorescent microspheres (1 μm, Polysciences) were added to all samples as standards and were calibrated daily using a SureCount Particle Count Standard (10 μm, Polysciences) bead solution. Flow cytometry data were not available for the dilution experiments conducted in 2009.

Bacteria were enumerated by staining 0.5 ml water samples with SybrGreen I (final concentration 5 μM) for 30-60 minutes in the dark. Samples were run at a threshold of 700 on FL1-H for 1 minute at a slow flow rate (14 μL min^{-1}, core diameter 10 μm). High and low nucleic acid content bacteria (High NA and Low NA, respectively) (Gasol et al. 1999) were differentiated by plotting FL3-H by FL1-H (Figure 3.2 a,b). Autotrophic particles were enumerated by running 0.5 ml water samples at a threshold of 700 on FL3-H for 3 minutes at a fast flow rate (66 μL min^{-1}, core diameter 22 μm). Different autotrophic assemblages were separated by plotting SSC-H (side scatter) by FL3-H. Size fractions: Nano = Nanophytoplankton (approx. 2 – 20 μm), Pico = Picoeukaryotes (approx. 1 – 2 μm) (Figure 3.2 c,d).

Microzooplankton enumeration

Microzooplankton (single-celled protists, 20 – 200 μm) were enumerated by preserving 250 – 500 ml water samples from the 100% whole water treatments at the initial and final time points with acid Lugol's (final concentration 6 – 8%). All samples were processed in our laboratory at the Virginia Institute of Marine Science. Microzooplankton were identified to major taxa, but some abundant or conspicuous forms were identified to genus or species level. Samples were first concentrated by settling in the collection jars for at least one week and then removing the top half of

supernatant. Aloricate ciliates, tintinnids, and the silicoflagellate *Dictyocha speculum* were enumerated by settling subsamples of the remaining half in 50 ml Utermöhl chambers, and dinoflagellates by settling subsamples in either 10 ml or 50 ml Utermöhl chambers. The entire contents (containing at least 100, but typically 200 – 500 cells of the most abundant taxa) were counted using an inverted microscope after a minimum 24-hour settling period (Utermöhl 1931). The silicoflagellate *Dictyocha speculum* was included in the analysis because live silicoflagellate cells have been observed to have pseudopodia extending from their spines, indicating heterotrophy (Martini 1977). Dinoflagellates were counted separately after clearing the sample with several drops of 5% sodium thiosulfate. Heterotrophic nanoflagellates (<20 μm) were not enumerated as the Utermöhl method severely underestimates their abundance (Davis and Sieburth 1982).

Heterotrophic dinoflagellates were distinguished from autotrophic dinoflagellates using epifluorescence microscopy, both by spot-checking live samples shipboard, and by filtration and DAPI staining for analysis back in our home laboratory. Samples (20-50 ml) were preserved with glutaraldehyde (final concentration 1%) and stained with 4'6' diamidino-2-phenylindole (DAPI), then subsequently filtered on 5 μm Nuclepore polycarbonate black membrane filters under gentle vacuum filtration (<200 mm Hg) shipboard. The filter was mounted on a slide and stored at -20°C until examination with epifluorescence microscopy in our home laboratory (Sherr and Sherr 1993a, Sherr et al. 1993). However, because mixotrophy is common among protists (Fenchel 2008), creating divisions between heterotrophs and autotrophs is often irrelevant. Dinoflagellates were

binned into size classes (i.e. small 20 – 50 μm) rather than identified by genus or species, and some autotrophs/mixotrophs were most likely included in these analyses.

Growth and grazing calculations

Grazing calculations represent the grazing of the entire microzooplankton community (20 – 200 μm, protozoans and tiny metazoans) as well as all protozoans <20 μm, (e.g., heterotrophic nanoflagellates). The dilution method is routinely used to estimate rates of herbivory by microzooplankton, and the assumptions of this method have been tested and proven valid for estimating rates of bacterivory as well (Tremaine and Mills 1987). Numerous studies have calculated bacterial growth and mortality rates using this method (Rivkin et al. 1999, Putland 2000, Anderson and Rivkin 2001, Tijdens et al. 2008, Pearce et al. 2010, 2011, Dupuy et al. 2011); therefore, rates of bacterivory were estimated. Growth and mortality (the latter assumed to be equivalent to microzooplankton grazing, hereafter referred to as grazing mortality) rates of phytoplankton and bacteria were estimated using the exponential model developed by Landry and Hassett (1982):

$$P_t = P_o e^{(\mu - g)t}$$

where P_t and P_o are chlorophyll (bacterial) concentrations at time t and time 0 and μ and g is the instantaneous coefficients of phytoplankton (bacterial) growth and mortality, respectively. The apparent growth rate (assuming $g = 0$) for each dilution was calculated according to the following equation (Landry and Hassett 1982):

$$\mu = 1/t \; ln(P_t/P_o)$$

The rate of change of phytoplankton (bacterial) density is linearly related to the dilution factor; therefore, the negative slope of the relationship is the mortality coefficient g and the y-intercept is the phytoplankton (bacterial) growth rate μ. The coefficients were determined by least-squares regression analysis of changing chlorophyll (bacterial) concentration at the $\alpha = 0.05$ level of significance. When the slope of the regression was not significant ($p > 0.05$, NS), rates were categorized as (i) zero grazing (flat line, no significant difference among growth rates at all dilution treatments determined by ANOVA, $p > 0.05$), or (ii) undetectable (scattered points, significant difference in growth rates among one or more dilution treatments determined by ANOVA, $p < 0.05$)..

For mortality rates statistically greater than zero, microzooplankton grazing pressure on initial phytoplankton and bacterial stock (P_i and B_i, respectively) and potential grazing pressure on primary and bacterial production (P_p and Bp, respectively) were calculated according to the following equations (Li et al. 2001):

$$P_i = 1 - e^{g} \times 100\%$$

$$P_p = (e^{\mu} - e^{(\mu-g)}) / (e^{\mu} - 1) \times 100\%$$

RESULTS

Phytoplankton and microzooplankton abundance

Chlorophyll-*a* concentrations at the beginning of each experiment ranged from 0.481 to 12.7 μg L^{-1} on the cruises in 2009 – 2011 (Table 3.1), and from 0.651 to 4.33 μg L^{-1} at Palmer Station (Table 3.2). Chlorophyll-*a* concentrations generally increased with increasing latitude in all three years (Table 3.1). Experiment 10, with a high chlorophyll-*a* concentration (7.40 μg L^{-1}, Table 3.1) was located in Marguerite Bay, historically a productivity hot-spot (Ducklow et al. 2012a). Phytoplankton assemblages in the northern part of the Peninsula were dominated by small autotrophs, while those in the southern part were dominated by large diatoms. Chlorophyll-*a* concentrations in experiments conducted across two months at the same location near Palmer Station (0.651 – 4.33 μg L^{-1}) did not vary as widely as those observed along the axis of the Peninsula. At Palmer Station, the higher chlorophyll-*a* concentrations in experiments in early February (Expts. P1 and P2, Table 3.2) are indicative of the latter part of the summer phytoplankton bloom, followed by decreased chlorophyll concentrations and then a small, secondary bloom in March (Figure 3.3).

On average, microzooplankton assemblages were numerically dominated by athecate dinoflagellates and aloricate ciliates (Figure 3.4), although on the LTER cruises, tintinnids were as abundant as the former two groups (Figure 3.4a). Tintinnid abundance was dominated by *Salpingella* spp., a genus comprised of generally small tintinnids, while larger tintinnids (i.e. *Laackmaniella* spp.) were much less abundant.

Microzooplankton abundance changed, sometimes substantially, over the course of the experiments. In each experiment where microzooplankton were enumerated (all

dilution experiments except those performed in 2009), at least one taxonomic group either increased or decreased by at least 25%. For example, in Experiments 8 and 9, all groups of microzooplankton more than doubled their abundances by the end of the experiments. In contrast, in Experiment P5 at Palmer Station on 26-Feb, all microzooplankton except the silicoflagellate decreased in abundance by more than half. In fact, a large ciliate bloom at the beginning of this experiment (39.1 $\times 10^3$ ind. L^{-1}) was reduced by one order of magnitude (final concentration 3.62 $\times 10^3$ ind. L^{-1}) by the end of the experiment (Appendix 5).

Phytoplankton growth and mortality

On the annual LTER cruises along the WAP in 2009 – 2011, phytoplankton mortality rates were significantly different than zero in only 3 of 12 experiments (Table 3.1). In these three experiments, pico-autotrophs and Total phytoplankton had moderate growth rates, and microzooplankton removed 62 – 71% and 89 – 116% of phytoplankton production, respectively (Table 3.1). In the experiments with non-significant grazing rates, 16 regressions (on all three phytoplankton size classes) were flat lines (indicating zero grazing rates), and 10 regressions were scattered points (indicating undetectable grazing rates) (see Table 3.1). In the nearshore waters close to Palmer Station in February – March 2011, phytoplankton grazing mortality rates were significantly different than zero in four of seven experiments (Table 3.2). In these four experiments, Total phytoplankton growth rates were 0.33 – 0.55 d^{-1} and grazing mortality rates were 0.10 – 0.31 d^{-1} (Figure 3.5a, Table 3.2). These rates were significantly lower than growth and grazing mortality of the smaller phytoplankton size classes (Pico + Nano) (0.52 – 0.99 d^{-1}

and 0.34 – 0.56 d^{-1}, respectively) (Figure 3.5a, Table 3.2, Student's t-test, $p < 0.01$). Phytoplankton growth rates were always higher than grazing mortality rates and microzooplankton removed 32 – 71% of phytoplankton production (Figure 3.5b, Table 3.2). Microzooplankton removed significantly more small phytoplankton (Pico + Nano) production (61-71%) compared to Total phytoplankton production (32-63%) (Student's t-test, $p < 0.05$). In the experiments with non-significant grazing rates, 8 regressions (on all three phytoplankton size classes) were flat lines (indicating zero grazing rates), and 4 regressions were scattered points (indicating undetectable grazing rates) (see Table 3.2).

A strong storm with wind gusts up to 60 knots occurred between experiments carried out on 11-Feb and 15-Feb. Before the storm, chlorophyll-*a* concentrations and phytoplankton growth rates were high and there was relatively high grazing mortality on all three size classes. Immediately after the storm (experiments conducted on 15-Feb and 16-Feb), phytoplankton growth and mortality rates were low or negligible and phytoplankton biomass was low (Figure 3.3, Figure 3.5).

Bacterial growth and mortality

On the LTER cruises in 2010 and 2011, microzooplankton grazing rates on either high nucleic acid content bacteria (High NA) or low nucleic acid content bacteria (Low NA) were significantly different than zero in all but one experiment (Table 3.3). In both years, growth and grazing mortality rates for High NA bacteria were higher compared to those for Low NA bacteria (Student's t-test, $p=0.07$ and 0.1, respectively). Growth and grazing mortality rates of High NA were 0.17 – 0.95 d^{-1} and 0.08 – 0.43 d^{-1}, respectively; growth and grazing mortality rates of Low NA were 0.03 – 0.17 d^{-1} and 0.06 – 0.20 d^{-1},

respectively (Figure 3.6a, Table 3.3). Interestingly, while growth and grazing mortality were higher for High NA bacteria, the calculated proportion of High or Low NA production removed by grazing did not necessarily follow the same pattern, as microzooplankton removed 52 – 134% of High NA production, and 100 – 412% of Low NA production (Figure 3.6, Table 3.3). Therefore, when growth and grazing of Low NA bacteria were detectable (in less than half of experiments), microzooplankton had a large grazing impact on Low NA bacterial production. For example, in Experiment 8, Low NA bacterial growth and grazing mortality rates were about half of the High NA rates, but microzooplankton removed a comparable amount of High NA and Low NA production (Figure 3.6, Table 3.3). In the experiments with non-significant grazing rates, 6 regressions (on all three bacterial types) were flat lines (indicating zero grazing rates), and 6 regressions were scattered points (indicating undetectable grazing rates) (see Table 3.3).

At Palmer Station, microzooplankton grazing rates on bacteria were significantly different than zero in all but one experiment (Table 3.4). Growth and grazing mortality rates of High NA were 0.18 – 0.36 d^{-1} and 0.09 – 0.34 d^{-1}, respectively; growth and grazing mortality rates of Low NA were 0.07 – 0.23 d^{-1} and 0.09 – 0.15 d^{-1}, respectively (Figure 3.7, Table 3.4). The high NA bacteria often had higher growth rates compared to Low NA bacteria. Microzooplankton removed 42 – 158% of High NA production and 51 – 141% of Low NA production (Figure 3.7b). In the experiments with non-significant grazing rates, 2 regressions (on all three bacterial types) were flat lines (indicating zero grazing rates), and 4 regressions were scattered points (indicating undetectable grazing rates) (see Table 3.4).

Although not as dramatic as the effect on phytoplankton growth and grazing rates, the storm also impacted the bacterial dynamics in the microbial food web. High NA bacterial mortality was significantly higher in the experiments directly after the storm compared to before the storm (Student's t-test, $p = 0.02$). This is in contrast to phytoplankton growth and mortality, which were lower after the storm.

Nutrient analysis

Nutrient analysis of water from the initial and final time points from the 100% whole water treatments in experiments indicate that dissolved inorganic nutrients (silicate, phosphate, nitrate + nitrite) were not limiting to phytoplankton growth in experiments in 2010 (Experiments 4-7) as nutrients were not depleted in any of the experimental bottles and the N:P ratios at the beginning and end of the experiments were not substantially different from the canonical Redfield ratio of N:P = 16:1 (Table 3.5; Redfield et al. 1963). This is in agreement with other nutrient consumption ratios that conform to the canonical Redfield ratios in the Southern Ocean (Hoppema and Goeyens 1999), although nutrient drawdown ratios can substantially differ from canonical ratios depending on phytoplankton assemblage composition (Arrigo et al. 2000, 2002). Si:P ratios at the beginning and end of the experiments were always well-above the typical ratio of 15:1, which has been documented in other regions of the Southern Ocean (Le Jehan and Tréguer 1983). Nutrients could have been limiting in several experiments in 2011, as phosphate was almost depleted in Experiments 10-12 (Table 3.5). Although low phosphate concentrations could be explained by the internal storage of phosphate by diatoms (Lund 1950, Tilman and Kilham 1976), any values under 0.1 μM PO_4 were

considered to be potentially limiting in these experiments. The elemental ratios from the coastal experiments at Palmer Station were more variable than those from experiments along the WAP in 2010 (Table 3.5), but the nutrients in these experiments were not depleted in any experimental bottles; thus, nutrient limitation likely did not affect the results of these experiments. In the few experiments with nutrient amendments, phytoplankton growth rates were not significantly different from those treatments without amendments (Student's t-test, $p > 0.05$). A few bacterial growth rates were significantly higher with inorganic nutrient enrichments compared to those without amendments (see Tables 3.3, 3.4).

Relationship between growth and grazing, and the effect of temperature on grazing

There was a significant positive linear relationship between phytoplankton growth and phytoplankton grazing mortality (Figure 3.8a), but growth and mortality rates were not balanced (slope $\neq 1$) because phytoplankton growth rates were usually higher than mortality rates (Tables 3.1 and 3.2). There was also a significant positive linear relationship between bacterial growth and bacterial grazing mortality (Figure 3.8b), although not as strong as the phytoplankton growth vs. mortality relationship. Bacterial growth and mortality rates were also not balanced (slope $\neq 1$).

We also considered the effect of temperature on microzooplankton grazing rates on phytoplankton and bacteria. Phytoplankton growth and grazing mortality rates significantly exponentially increased at higher temperatures ($p = 0.03$ and 0.03, respectively), although water temperature was a poor indicator of phytoplankton growth and grazing mortality ($r^2 = 0.21$ and 0.22, respectively) (Figure 3.9a). We found a trend

of increasing bacterial growth and grazing mortality with increasing temperatures, but these were not statistically significant ($p > 0.05$) (Figure 3.9b).

DISCUSSION

Phytoplankton growth and grazing mortality

Phytoplankton grazing mortality rates along the WAP (NS – 0.31 d^{-1}, average of significant mortality rates = 0.24 d^{-1}, NS = not significant) and in the nearshore waters near Palmer Station (NS – 0.56 d^{-1}, average of significant mortality rates = 0.30 d^{-1}) were generally lower than average phytoplankton grazing mortality rates reported in tropical and temperate habitats (0.72 and 0.69 d^{-1}, respectively, Calbet and Landry 2004), but were near the average for polar waters (0.44 d^{-1}, Calbet and Landry 2004). While there are no published studies of phytoplankton mortality rates using the dilution method in the LTER study region along the WAP, Burkill et al. (1995) and Tsuda and Kawaguchi (1997) report results of phytoplankton grazing mortality in the Bellingshausen Sea (to the south of our study area) and near King George Island (at the tip of the Antarctic Peninsula), respectively. They report phytoplankton mortality rates of 0.03 – 0.52 d^{-1} (Burkill et al. 1995) and NS – 0.29 d^{-1} (Tsuda and Kawaguchi 1997), which are in the same range as the grazing mortality rates presented here (NS – 0.56 d^{-1}). Other studies in the Southern Ocean have calculated wide ranges of phytoplankton growth (NS – 2.6 d^{-1}) and grazing mortality rates (NS – 2.36 d^{-1}), and most also report experiments with mortality rates that are not significantly different than zero.

Phytoplankton growth rates often exceeded microzooplankton grazing rates in this extremely productive season. As measured along the LTER grid, microzooplankton grazed 62 – 116% (average = 85%) of primary production, and nearshore Palmer Station grazed 32 – 71% (average = 55%) of phytoplankton production. In our study phytoplankton growth and grazing mortality were significantly positively correlated, but

were not balanced because phytoplankton growth rates were usually higher than mortality rates. In fact, mortality rates only exceeded growth rates in one experiment (Experiment 6, Total phytoplankton size class, Table 3.1). Tsuda and Kawaguchi (1997) found that phytoplankton growth and grazing mortality were often balanced in their experiments at the tip of the Antarctic Peninsula. Pearce et al. (2008, 2010) concluded that microzooplankton often removed >100% of primary production and were key to controlling phytoplankton growth near Davis Station in East Antarctica, especially at sites near the ice edge. Therefore, although microzooplankton can exert considerable grazing pressure on phytoplankton at certain times and locations, in this study, phytoplankton production exceeded microzooplankton grazing, which could partially explain the large phytoplankton blooms that occur in the WAP.

Phytoplankton mortality rates were significantly different than zero in 25% (3 of 12) of dilution experiments on the LTER cruises and in 57% (4 of 7) of dilution experiments conducted in the nearshore waters close to Palmer Station. Of the non-significant regressions more than half were flat lines, indicating zero grazing (16 of 26 regressions on cruises and 8 of 12 regressions near Palmer Station, Tables 3.1 and 3.2). The low grazing mortality rates (compared to temperate and tropical habitats), in addition to the fraction of experiments in which grazing was not statistically significant (zero or undetectable) in this study (especially on the LTER cruises) agree with the results presented by Caron et al. (2000). They calculated significant phytoplankton mortality rates in 26% (9 of 34) of experiments conducted in the Ross Sea and when significant, mortality rates were low ($<0.26\ d^{-1}$). They attributed the low and non-significant grazing mortality rates partially to extremely low temperatures, which likely limited

microzooplankton herbivory, as well as to phytoplankton community composition, which can influence microzooplankton grazing.

We compared microzooplankton to macrozooplankton grazing impact in the nearshore and shelf regions of the WAP and found that microzooplankton grazing pressure on phytoplankton was higher than macrozooplankton grazing. Grazing rates of the five dominant macrozooplankton in the WAP (krill *Euphausia superba, Euphausia crystallorophias,* and *Thysanöessa macrura*; the pteropod *Limacina helicina*; and the salp *Salpa thompsoni*) were determined separately on the LTER cruises in 2009 and 2010. In nearshore and shelf waters (where dilution experiments were conducted), the dominant macrozooplankton removed 0 – 3% of primary productivity (Bernard et al. 2012), considerably lower than that removed by microzooplankton (NS – 116%, significant rates usually between 30 – 70%). The highest grazing pressure by macrozooplankton (up to 641% of primary productivity) was associated with large salp blooms which were usually located offshore near the shelf break (Bernard et al. 2012). Although no dilution experiments were conducted off shelf in oceanic waters, macrozooplankton grazing pressure on phytoplankton could exceed that of microzooplankton in the offshore region where salps form dense blooms. In the nearshore and shelf waters of the WAP, macrozooplankton plus microzooplankton grazing was not sufficient to control phytoplankton growth.

Environmental and experimental considerations for measurement of phytoplankton growth and microzooplankton grazing

A number of factors can affect microzooplankton grazing rates, including phytoplankton and microzooplankton assemblage structure, nutrient limitation, and predation by larger zooplankton (Landry and Hassett 1982, Weisse et al. 1994, First et al. 2007). *Phaeocystis antarctica,* a colonial prymnesiophyte that is widely distributed in the Southern Ocean, can possibly deter protozoan grazers by forming large colonies (Weisse et al. 1994, Brussaard et al. 1996). However, Brussaard et al. (2005) observed microzooplankton actively moving in and out of colonies *Phaeocystis*, suggesting some microzooplankton taxa may be able to efficiently consume *Phaeocystis* colonies (Shields and Smith Jr. 2008). In the Ross Sea *P. antarctica* colonies and diatoms dominated the samples collected coincident with dilution experiments, which could have contributed to low grazing mortality rates observed (Caron et al. 2000). However, large colonies of *P. antarctica* were not encountered in our study. Single-celled *Phaeocystis* spp. are palatable to protozoan grazers (Admiraal and Venekamp 1986, Weisse et al. 1994) and occur in the coastal regions of the WAP (Garibotti et al. 2003). Therefore, the presence of *P. antarctica* would not have affected microzooplankton grazing rates in our study. Large diatoms were abundant, especially in the southern part of our study region (Montes-Hugo 2009). Due to their varied feeding strategies (Hansen and Calado 1999, Jeong 1999, Stoecker 1999), dinoflagellates may be the main consumers of large diatoms, as diatoms could be too large and have spines or other grazing deterrents that prevent their consumption by herbivorous ciliates (Verity and Villareal 1986). Overall microzooplankton grazing rates could have been reduced by large diatoms in these

experiments. The lowest grazing rates were calculated on the Total phytoplankton size class (which would include large diatoms) while the highest grazing rates were calculated on the smaller phytoplankton size classes (see discussion below on "selective feeding"), suggesting the large diatoms negatively affected microzooplankton grazing rates.

Macronutrients (silicate, nitrate + nitrite, phosphate) were not added to our experiments as phytoplankton growth is generally not limited by macronutrients in the Southern Ocean (Martin 1990). However, in a few experiments in productive areas, macronutrients could have been limiting. In 2011, the non-significant phytoplankton mortality rates in Experiments 10-12 could have been due to nutrient limitation of phytoplankton growth in experimental bottles. These experiments were done near Marguerite Bay (Experiment 10) and in the south near Charcot Island (Experiments 11-12), areas with high productivity and phytoplankton biomass. Nutrient limitation did not confound results of other experiments (Table 3.5).

Although grazer dynamics are an inherent part of dilution experiments, substantial changes in the grazer community could affect grazing mortality rates (First et al. 2007). Changes in microzooplankton assemblages can be due to predation, however larger zooplankton are excluded from dilution experiments to prevent predation on phytoplankton or microzooplankton. Excluding these predators can avoid complicated trophic cascade effects, but also could cause overestimation of microzooplankton grazing rates by removing microzooplankton from predation pressure. However, microzooplankton can still consume other microzooplanktonic grazers (Dolan 1991), further complicating interpretation of dilution experiment results.

These complicated trophic dynamics were apparent in some of our experiments as over the course of some experiments microzooplankton abundance decreased or increased. For example, in Experiment P5 on 26-Feb, all microzooplankton except the silicoflagellate decreased in abundance by more than half. There was a very large ciliate bloom at the beginning of the experiment (39,080 ind. L^{-1}) that was reduced by one order of magnitude by the end of the experiment. This large decrease in microzooplankton grazers in this experiment could indicate complicated trophic dynamics and might have caused phytoplankton mortality rates in this experiment to be zero or undetectable. On the other hand, in Experiments 8 and 9 all microzooplankton taxonomic groups more than doubled their populations in the whole water treatments by the end of the experiments (Appendix 5). Other studies have reported varying microzooplankton growth in different dilution treatments (Dolan et al. 2000) which could result in nonlinear feeding responses and non-significant results. Although we can only speculate as grazers were only enumerated in the 100% whole water treatments, microzooplankton growth could have differed among treatments, contributing to the non-significant results.

In the experiments where phytoplankton grazing mortality rates were significantly different than zero, microzooplankton abundance was substantially higher only at the end of Experiment P1 at Palmer Station (athecate dinoflagellate abundance increased 3 times). This could have caused an overestimate of microzooplankton grazing rates. In all other experiments with statistically significant grazing rates, abundances of major taxa of microzooplankton did not change substantially.

Bacterial growth and mortality

Microzooplankton exerted substantial grazing pressure on bacteria, often removing >100% of bacterial production. This is in contrast to grazing control on phytoplankton assemblages, as microzooplankton removed >100% of phytoplankton production in only one experiment. There are limited terrestrial sources of dissolved organic matter (DOM) in Antarctic waters; therefore, bacterioplankton in coastal waters depend on phytoplankton production for organic matter and should be coupled with phytoplankton populations. In a recent time series analysis (2003 – 2011) WAP bacterial production during the summer (measured on the annual LTER cruise in January, as well as at Palmer Station during late October – March) was correlated with chlorophyll-*a* and, on average, chlorophyll explained approximately 50% of the variation in bacterial production as measured by ^{3}H-leucine incorporation rates (Ducklow et al. 2012b). Although bacterioplankton are more abundant in summer compared to winter in the WAP, they do not form as conspicuous of an annual bloom as they do in the Ross Sea (Ducklow et al. 2001); thus, WAP bacterial abundances are comparatively constant (Ducklow et al. 2012a). The high grazing pressure on bacteria determined in this study could effectively crop bacterial production, helping explain the relatively constant bacterial abundances in this region and why bacterial production is not more tightly coupled with chlorophyll.

Our calculations of bacterial growth and grazing mortality, as well as high grazing pressure on bacterial production, are corroborated in previous studies conducted in the Southern Ocean. Anderson and Rivkin (2001) reported bacterial growth (0.14 – 0.68 d^{-1}) and grazing mortality rates (0.04 – 0.58 d^{-1}) in McMurdo Sound (Ross Sea) in the

spring/summer, similar to the rates presented in our study (growth: NS – 0.95 d^{-1}, mortality: NS – 0.43 d^{-1}). Their study captured seasonal fluctuations in bacterial mortality rates and concluded that at times, microzooplankton can consume nearly 100% of bacterial production, while at other times grazing mortality is negligible (Anderson and Rivkin 2001). Bacterial growth and mortality rates were calculated off East Antarctica in the South West Indian Ocean sector of the Southern Ocean. The rates covered wide ranges (Pearce et al. 2010) and were generally higher than those reported here, as well as in other Southern Ocean studies. Microzooplankton off East Antarctica could remove >100% of bacterial production (Pearce et al. 2010), which is consistent with our findings. In the only other study calculating bacterial growth and mortality with the dilution method in the WAP (north of our study area), bacterial mortality rates were not significantly different than zero in one experiment, and the response of the bacterial community to dilution was nonlinear in the other experiment (Bird and Karl 1999).

Using data compiled from the Arctic and Antarctic, Anderson and Rivkin (2001) found a significant positive correlation between bacterial growth and grazing mortality and that grazing losses generally equaled bacterial growth. We also found a significant positive correlation between bacterial growth and mortality, but these rates were not balanced. Grazing mortality was often higher than growth, resulting in removal of >100% of bacterial production. This suggests that microzooplankton can exert high grazing pressure on bacteria along the WAP during the summer.

Selective feeding

Selective feeding by microzooplankton is common and widespread (Stoecker et al. 1981, Tillmann 2004, Strom et al. 2007), and preferential grazing on fast-growing phytoplankton taxa has been documented in the North Atlantic (Gaul and Antia 2001). At Palmer Station, grazing mortality and the fraction of primary production removed was significantly higher on the smaller phytoplankton size classes (Pico + Nano, which also had significantly higher growth rates), compared to the Total phytoplankton size class, which included large diatoms. This could indicate selective feeding on smaller phytoplankton, and/or preferential grazing on the more actively growing phytoplankton size classes in the assemblage. Large diatoms can be difficult for some microzooplankton taxa, especially ciliates, to consume (Verity and Villareal 1986), which could explain the lower grazing mortality rates on the Total phytoplankton size class. An alternative explanation is that the Total phytoplankton size class had lower growth rates compared to the smaller phytoplankton (Pico and Nano), and microzooplankton inherently have lower grazing rates when phytoplankton growth rates are lower (Figure 3.8a).

In addition to selective feeding on phytoplankton, the grazers in these experiments also appeared to selectively graze the High NA bacteria compared to the Low NA bacteria. In more temperate waters, High NA bacteria are the larger, more actively dividing cells in the population (Gasol et al. 1999) and selective grazing by protists on these larger, motile, actively growing cells has been documented (Sherr et al. 1992, Gonzalez et al. 1993, del Giorgio et al. 1996). In these experiments, High NA bacteria had higher growth rates than Low NA bacteria. Grazing mortality rates were also higher compared to Low NA bacteria. In all but one experiment on the LTER cruises in 2011

(Experiment 8), growth and grazing mortality rates were not statistically significant (zero or undetectable) for Low NA bacteria. Collectively these results suggest that microzooplankton selectively grazed High NA bacteria, the more actively growing bacterial cells in these experiments (Sherr et al. 1992).

Physical forcing and the effect of temperature on microbial food web dynamics

The strong wind storm that occurred between Experiments P2 and P3 at Palmer Station might have altered the microbial food web dynamics at this location. Chlorophyll concentrations and phytoplankton growth rates decreased after the storm, which appeared to affect microzooplankton grazing capacity on phytoplankton. Several studies have documented the effect of strong storms on phytoplankton dynamics and microzooplankton grazing in lower latitude systems (Wetz et al. 2006, Zhou et al. 2011) and decreases in microzooplankton biomass have been documented in coastal systems after strong storms (Zhang and Wang 2000, Lawrence et al. 2004). There was no evidence of significant changes in microzooplankton biomass after the storm in our study (see Appendix 5). It is more likely that the decrease in phytoplankton biomass and growth rates after the storm affected microzooplankton grazing rates, as microzooplankton appeared to selectively graze the faster growing phytoplankton cells in the assemblage (see discussion above). In contrast, microzooplankton had higher grazing pressure on High NA bacteria after the storm. Other studies suggest microzooplankton communities are able to shift between herbivory and bacterivory (Anderson and Rivkin 2001); although based on only one storm event, these results suggest that the

microzooplankton community might have collectively shifted its major prey item from phytoplankton (before the storm) to bacteria (after the storm).

Temperature affects microzooplankton physiological rates (Sherr et al. 1988, Choi and Peters 1992, Sherr and Sherr 1994, Rose et al. 2008), and several studies have suggested that low temperatures constrain microzooplankton growth and grazing rates, accounting for the often low phytoplankton mortality rates reported in the Southern Ocean (Caron et al. 2000, Rose and Caron 2007). We found a significant ($p < 0.05$) exponential relationship between temperature and phytoplankton growth and mortality rates, although only 22 and 21% of the variability in phytoplankton growth and mortality rates, respectively, could be explained by water temperature. The relationship between temperature and bacterial mortality rates was not statistically significant. Therefore, water temperature is a poor indicator of phytoplankton and bacterial growth and mortality. The range of experimental temperatures in this study was narrow (<4°C), and with more experiments conducted at the higher end of the range. The temperature range was possibly not sufficient to result in large differences in microzooplankton grazing rates with increasing temperature. Therefore, due to the different sample sizes at different temperatures, as well as the variability and small range of experimental temperatures, we did not find robust evidence for an effect of temperature on microzooplankton grazing rates. Other studies in polar waters have found no effect of temperature on rates of microzooplankton grazing on phytoplankton (Froneman and Perissinotto 1996b, Tsuda and Kawaguchi 1997, Pearce et al. 2010). Tsuda and Kawaguchi (1997) found a positive correlation between phytoplankton growth and temperature in the northern Antarctic Peninsula, but no effect of temperature on microzooplankton grazing. These authors

concluded that temperature might be an important factor that creates variability in phytoplankton growth rates, while not necessarily affecting microzooplankton grazing rates.

SUMMARY AND CONCLUSION

Microzooplankton are an integral part of microbial food webs and have the capacity to consume a large proportion of phytoplankton and bacterial production. We report the first results of microzooplankton grazing rates on phytoplankton and bacteria along the WAP, a region of rapid climate change. Phytoplankton grazing mortality rates were generally lower than average grazing mortality rates reported in tropical and temperate waters, but were comparable to the average reported for polar waters (Calbet and Landry 2004). Phytoplankton growth rates exceeded grazing mortality rates in all but one experiment. Microzooplankton exert higher grazing pressure on phytoplankton compared to the dominant macrozooplankton in WAP shelf and coastal waters (Bernard et al. 2012). However, combined grazing by micro- and macrozooplankton was usually not equivalent to primary production, which could partially explain the large phytoplankton blooms that occur along the WAP in this extremely productive season. While grazing pressure was not enough to control phytoplankton blooms that occur during the summer, microzooplankton exerted substantial grazing control on bacterioplankton, which could explain the relatively constant bacterial abundances during the summer in the WAP (Ducklow et al. 2012a).

In these experiments microzooplankton preferentially grazed on the smaller, faster-growing phytoplankton cells, as well as the High NA bacteria, which were the more actively dividing cells in the bacterial assemblages. In this respect, microzooplankton potentially altered phytoplankton and bacterial assemblages by cropping the fastest growing groups. Physical factors, such as water temperature or mixing (from storms), may also have affected microbial food web dynamics, although

more research is needed to determine their specific effects on microzooplankton grazing. Although previous studies found an effect of temperature on microzooplankton growth rates (and subsequently grazing rates, Rose and Caron 2007), given the lack of correlation between temperature and microzooplankton grazing rates presented in many studies in the Southern Ocean, it seems more reasonable to conclude that temperature might be an important factor that creates variability in phytoplankton growth rates, while not necessarily affecting microzooplankton grazing rates. Also, the fraction of primary production removed in these experiments (when grazing rates were significantly different than zero) as well as other experiments in the Southern Ocean (see Table 3.6) was comparable to the fraction of primary production removed in tropical and temperate habitats (Calbet and Landry 2004), suggesting that microzooplankton grazing impact at colder temperatures is comparable to their impact at higher temperatures.

Phytoplankton and bacterial growth and grazing mortality were very dynamic in this region. While this study provides a reference point for microzooplankton grazing impact along the WAP in summer, further research is needed to clarify their trophic role during different seasons and to better define the specific effect of temperature on microzooplankton grazing rates. Incorporating these results into food web and biogeochemical models will substantially improve our ability to predict changes in the WAP ecosystem with changing climate.

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Table 3.1. Phytoplankton growth and grazing mortality rates calculated from dilution experiments performed at sites along the Western Antarctic Peninsula in January 2009, 2010 and 2011. Experiment numbers correspond with stations shown in Figure 3.1. 'Grid' is the Palmer LTER station identification (Waters and Smith 1992). Lat = Latitude. Long = Longitude. Chl-*a* = the initial chlorophyll-*a* concentration determined fluorometrically from the 100% treatment of each experiment, ± S.E. Size fraction: Total = as measured by Chl-*a* extraction (filtration on GF/F filters, nominal pore size 0.7 μm); Nano = Nanophytoplankton (approx. 2 – 20 μm) and Pico = Picoeukaryotes (approx. 1 – 2 μm); size fractions were determined using flow cytometry in 2010 and 2011 but was not available for experiments done in 2009. μ = phytoplankton growth rate, g = grazing mortality rate, P_i and P_p are the potential microzooplankton grazing pressure on initial phytoplankton stock and primary production, respectively. * = $p < 0.01$. Rates that were not statistically significant ($p > 0.05$) were categorized as (0) zero grazing (flat line, no significant difference among growth rates at all dilutions determined by ANOVA, $p > 0.05$), or (+) undetectable (scattered points, significant difference in growth rates among one or more dilution determined by ANOVA, $p < 0.05$). P_i and P_p were not calculated for the non-significant rates. Water temperature was measured by CTD at the location where the water was collected for each experiment.

Expt #	Date	Grid	Lat (°S) Long (°W)	Chl-*a* (μg L^{-1})	Water temp. (°C)	Size fraction	μ (d^{-1})	g (d^{-1})	P_i (%)	P_p (%)	r^2
1	08-Jan-09	590.130	64.46, 65.94	0.481 ± 0.026	1.2	Total^0	0.20	0.02	-	-	0.02
2	19-Jan-09	167.-033	68.20, 70.00	1.26 ± 0.048	1.0	Total$^+$	0.22	-0.02	-	-	0.00
3	26-Jan-09	-100.000	69.50, 75.51	1.83 ± 0.011	0.18	Total	0.13	0.12	11	89	0.70*
4	08-Jan-10	585.135	64.47, 66.20	0.851 ± 0.058	0.14	Total$^+$	-0.11	-0.06	-	-	0.07
						Nano^0	-0.08	0.02	-	-	0.00
						Pico^0	0.08	0.15	-	-	0.06
5	18-Jan-10	167.-033	68.20, 70.00	1.56 ± 0.148	0.44	Total^0	-0.09	-0.10	-	-	0.03
						Nano$^+$	0.26	0.09	-	-	0.24
						Pico	0.50	0.28	24	62	0.53*
6	26-Jan-10	-121.-024	69.82, 75.55	1.24 ± 0.025	-1.60	Total	0.22	0.26	23	116	0.41*
						Nano^0	0.26	0.09	-	-	0.08
						Pico	0.46	0.31	26	71	0.51*
7	27-Jan-10	-100.000	69.50, 75.51	4.53 ± 0.378	-0.70	Total^0	0.19	0.09	-	-	0.23
						Nano^0	0.53	0.04	-	-	0.01
						Pico$^+$	0.39	0.07	-	-	0.06
8	11-Jan-11	600.040	64.93, 64.40	0.845 ± 0.101	0.90	Total$^+$	0.05	-0.17	-	-	0.70
						Nano^0	0.25	-0.06	-	-	0.08
						Pico^0	0.24	-0.05	-	-	0.08

Expt #	Date	Grid	Lat (°S) Long (°W)	Chl-*a* (μg L^{-1})	Water temp. (°C)	Size fraction	μ (d^{-1})	g (d^{-1})	P_i (%)	P_p (%)	r^2
9	12-Jan-11	600.040	64.93, 64.40	1.64 ± 0.116	0.90	$Total^+$	0.28	0.13	-	-	0.35
						$Nano^0$	0.39	0.05	-	-	0.03
						$Pico^0$	0.03	0.04	-	-	0.03
10	18-Jan-11	221.-019	67.77, 69.27	7.40 ± 1.07	0.95	$Total^+$	0.13	0.05	-	-	0.08
						$Nano^0$	0.47	0.01	-	-	0.01
						$Pico^0$	-0.05	0.04	-	-	0.01
11	29-Jan-11	-158.-034	70.09, 76.15	4.13 ± 0.146	0.88	$Total^+$	0.18	0.01	-	-	0.01
						$Nano^0$	0.44	0.11	-	-	0.06
						$Pico^+$	0.23	1.00	-	-	0.58
12	29-Jan-11	-161.-047	69.79, 76.17	12.7 ± 0.269	-0.70	$Total^0$	0.16	0.02	-	-	0.02
						$Nano^0$	0.27	0.03	-	-	0.01
						$Pico^+$	-0.21	0.21	-	-	0.02

Table 3.2. Phytoplankton growth and grazing mortality rates calculated from dilution experiments conducted near Palmer Station (64.78°S, 64.04°W) in February – March 2011. Chl-*a* = the initial chlorophyll-*a* concentration determined fluorometrically from the 100% treatment of each experiment, ± S.E. Size fraction: Total = as measured by chl-*a* extraction (filtration on GF/F filters, nominal pore size 0.7 μm); Nano = Nanophytoplankton (approx. 2 – 20 μm) and Pico = Picoeukaryotes (approx. 1 – 2 μm); size fractions were determined using flow cytometry. μ = phytoplankton growth rate, g = grazing mortality rate, P_i and P_p are the potential microzooplankton grazing pressure on initial phytoplankton stock and primary production, respectively. * = $p < 0.05$, ** = $p < 0.001$. Rates that were not statistically significant ($p > 0.05$) were categorized as (0) zero grazing (flat line, no significant difference among growth rates at all dilutions determined by ANOVA, $p > 0.05$), or (+) undetectable (scattered points, significant difference in growth rates among one or more dilution determined by ANOVA, $p < 0.05$). P_i and P_p were not calculated for the non-significant rates. Water temperature was measured by CTD at the location where the water was collected for each experiment.

Expt. #	Date	Chl-*a* (μg L^{-1})	Water temp. (°C)	Size fraction	μ (d^{-1})	g (d^{-1})	P_i (%)	P_p (%)	r^2
P1	09-Feb-11	4.33 ± 0.213	1.5	Total	0.38	0.12	11	36	0.43*
				Nano	0.81	0.41	34	61	0.73**
				Pico	0.67	0.36	30	62	0.52*
P2	11-Feb-11	2.25 ± 0.147	1.2	Total	0.55	0.31	27	63	0.88**
				Nano	0.99	0.56	43	68	0.65**
				Pico	0.83	0.42	34	61	0.65**
P3	15-Feb-11	0.651 ± 0.009	1.2	Total	0.33	0.11	10	37	0.46*
				Nano^0	0.62	-0.16	-	-	0.39
				Pico$^+$	0.46	-0.18	-	-	0.41
P4	16-Feb-11	0.706 ± 0.002	1.3	Total^0	0.32	0.02	-	-	0.05
				Nano^0	0.79	0.05	-	-	0.03
				Pico^0	0.41	-0.19	-	-	0.29
P5	26-Feb-11	0.917 ± 0.034	1.1	Total$^+$	0.27	-0.08	-	-	0.24
				Nano$^+$	0.50	0.12	-	-	0.16
				Pico^0	0.76	0.18	-	-	0.28
P6	15-Mar-11	1.37 ± 0.072	1.5	Total	0.35	0.10	10	32	0.81**
				Nano	0.52	0.34	29	71	0.75**
				Pico^0	0.52	0.13	-	-	0.20

Expt. #	Date	Chl-*a* (μg L^{-1})	Water temp. (°C)	Size fraction	μ (d^{-1})	g (d^{-1})	P_i (%)	P_p (%)	r^2
P7	19-Mar-11	1.38 ± 0.021	1.2	$Total^0$	0.46	0.01	-	-	0.01
				$Nano^0$	0.51	0.05	-	-	0.03
				$Pico^+$	0.36	-0.30	-	-	0.49

Table 3.3. Bacterial growth and grazing mortality rates calculated from dilution experiments conducted at sites along the Western Antarctic Peninsula (WAP) in January 2010 and 2011. Experiment numbers correspond with stations shown in Figure 3.1. Bacteria type: Total = total bacterial assemblage, High NA = high nucleic acid content bacteria, Low NA = low nucleic acid content bacteria. Bacterial types were determined using flow cytometry. k = bacterial growth rate, g = grazing mortality rate, B_i and P_p are the potential microzooplankton grazing pressure on initial bacterial stock and production, respectively. * = $p < 0.05$, ** = $p < 0.001$. Rates that were not statistically significant ($p > 0.05$) were categorized as (0) zero grazing (flat line, no significant difference among growth rates at all dilutions determined by ANOVA, $p > 0.05$), or (+) undetectable (scattered points, significant difference in growth rates among one or more dilution determined by ANOVA, $p < 0.05$). P_i and P_p were not calculated for the non-significant rates. See Table 3.1 for Palmer LTER station identification and locations, as well as environmental conditions. Experiments in 2009 were not analyzed for bacterial growth and mortality rates. Bacterial types in italics indicate bacterial growth rates were significantly higher ($p < 0.05$) with nutrient enrichments compared to those without amendments.

Expt. #	Date	Bacteria type	k (d^{-1})	g (d^{-1})	B_i (%)	B_p (%)	r^2
4	08-Jan-10	Total	0.04	0.09	9	208	0.50*
		High NA^0	0.06	0.06	-	-	0.23
		Low NA	0.03	0.13	12	412	0.84**
5	18-Jan-10	$Total^0$	0.11	0.06	-	-	0.09
		High NA	0.22	0.19	17	88	0.55*
		Low NA^+	-0.1	-0.20	-	-	0.51
6	26-Jan-10	$Total^0$	0.09	0.04	-	-	0.07
		High NA^0	0.10	0.03	-	-	0.02
		Low NA	0.06	0.06	6	100	0.30*
7	27-Jan-10	$Total^0$	0.11	0.04	-	-	0.02
		High NA^+	0.18	0.10	-	-	0.08
		Low NA^+	0.02	0.01	-	-	0.01
8	11-Jan-11	Total	0.27	0.34	29	122	0.94**
		High NA	0.29	0.41	34	134	0.91**
		Low NA	0.17	0.20	18	116	0.93**
9	12-Jan-11	Total	0.15	0.16	15	106	0.54**
		High NA	0.24	0.25	22	104	0.74**
		Low NA^0	-0.01	0.03	-	-	0.07
10	18-Jan-11	Total	0.82	0.38	32	56	0.94**
		High NA	0.95	0.43	35	61	0.93**
		Low NA^+	0.69	0.37	-	-	0.27
11	29-Jan-11	Total	0.31	0.24	21	80	0.86**
		High NA	0.30	0.17	16	60	0.79**
		Low NA^+	0.19	0.85	-	-	0.46
12	29-Jan-11	*Total*	0.16	0.08	8	52	0.39*
		High NA	0.17	0.08	8	52	0.37*
		Low NA^+	-0.05	0.03	-	-	0.03

Table 3.4. Bacterial growth and grazing mortality rates calculated from dilution experiments conducted near Palmer Station (64.78°S, 64.04°W) in February – March 2011. Bacteria type: Total = total bacterial assemblage, High NA = high nucleic acid content bacteria, Low NA = low nucleic acid content bacteria. Bacterial types were determined using flow cytometry. k = bacterial growth rate, g = grazing mortality rate, B_i and P_p are the potential microzooplankton grazing pressure on initial bacterial stock and production, respectively. High NA = high nucleic acid content bacteria, Low NA = low nucleic acid content bacteria. * = $p < 0.05$, ** = $p < 0.001$. Rates that were not statistically significant ($p > 0.05$) were categorized as (0) zero grazing (flat line, no significant difference among growth rates at all dilutions determined by ANOVA, $p > 0.05$), or (+) undetectable (scattered points, significant difference in growth rates among one or more dilution determined by ANOVA, $p < 0.05$). P_i and P_p were not calculated for the non-significant rates. See Table 3.2 for environmental conditions. Bacterial types in italics indicate bacterial growth rates were significantly higher ($p < 0.05$) with nutrient enrichments compared to those without amendments.

Expt. #	Date	Bacteria type	k (d^{-1})	g (d^{-1})	B_i (%)	B_p (%)	r^2
P1	09-Feb-11	Total	0.14	0.18	16	126	0.83**
		High NA	0.18	0.21	19	115	0.78**
		Low NA	0.07	0.10	10	141	0.90**
P2	11-Feb-11	Total	0.31	0.17	16	59	0.56*
		High NA	0.36	0.20	18	60	0.57*
		Low NA	0.23	0.11	10	51	0.44*
P3	15-Feb-11	*Total*	0.18	0.26	23	139	0.83**
		High NA	0.21	0.34	29	152	0.79**
		Low NA	0.14	0.15	14	107	0.91**
P4	16-Feb-11	Total	0.16	0.23	21	139	0.92**
		High NA	0.19	0.32	27	158	0.88**
		Low NA	0.13	0.15	14	114	0.96**
P5	26-Feb-11	Total^{0}	0.26	0.30	-	-	0.05
		High NA^{+}	0.35	0.07	-	-	0.25
		Low NA^{+}	0.07	-0.08	-	-	0.24
P6	15-Mar-11	Total	0.11	0.05	5	47	0.30*
		High NA^{0}	0.15	0.03	-	-	0.13
		Low NA	0.11	0.09	9	83	0.46*
P7	19-Mar-11	Total^{+}	0.13	0.03	-	-	0.08
		High NA	0.23	0.09	9	42	0.45*
		Low NA^{+}	0.07	-0.01	-	-	0.01

Table 3.5. Nutrient concentrations and elemental ratios in control treatments at the beginning and end of 72 hour dilution experiments. Calculated from nutrient analyses of initial and final time points of 100% whole water treatments in each dilution experiment done on the annual LTER cruises in January 2010 and 2011, and at Palmer Station in February – March 2011. Si = μM silicate (SiO_4), N = μM nitrate (NO_3) + nitrite (NO_2), P = μM phosphate (PO_4). Nutrient concentrations = mean ± S.E. Initial time point, n=2. Final time point, n=3. No S.E. recorded, n=1. **Bold** = possible nutrient limitation, (values under 0.1 μM PO_4 considered to be potentially limiting). * = non-significant nutrient uptake rate.

Expt. #	Date	Time point	Si (μM)	N (μM)	P (μM)	N:P	ΔN:ΔP
4	08-Jan-10	Initial	64.9	27.0	1.8	15:1	
		Final	43.1 ± 7.6	18.7 ± 4.5	1.2 ± 0.2	16:1	13:1
5	18-Jan-10	Initial	71.5	23.3	1.4	16:1	
		Final	66.9 ± 6.8	20.8 ± 2.2	1.3 ± 0.1	16:1	22:1
6	26-Jan-10	Initial	81.1	28.6	1.9	15:1	
		Final	78.2 ± 0.9	28.0 ± 0.3	1.7 ± 0.01	16:1	6:1
7	27-Jan-10	Initial	49.7	11.1	0.7	15:1	
		Final	55.6 ± 0.9	7.3 ± 0.3	0.5 ± 0.0	15:1	16:1
8	11-Jan-11	Initial	sample lost				
		Final	52.3 ±0.3	21.0 ± 0.3	1.2 ± 0.01	18:1	-
9	12-Jan-11	Initial	62.1 ± 1.2	21.8 ± 0.9	1.3 ± 0.0	17:1	
		Final	61.0 ± 1.0	21.0 ± 0.4	1.2 ± 0.01	18:1	10:1
10	18-Jan-11	Initial	28.6 ± 3.7	4.3 ± 1.0	0.2 ± 0.0	23:1	
		Final	**28.1 ± 3.7**	**1.7 ± 0.3**	**0.07 ± 0.0**	**25:1**	**23:1**
11	29-Jan-11	Initial	34.0 ± 1.8	4.6 ± 0.5	0.2 ± 0.0	26:1	
		Final	**37.7 ± 1.6**	**2.5 ± 0.5**	**0.04 ± 0.0**	**40:1**	**15:1**
12	29-Jan-11	Initial	38.8 ± 2.0	7.5 ± 0.1	0.2 ± 0.0	38:1	
		Final	**38.9 ± 1.3**	**1.5 ± 0.3**	**0.03 ± 0.0**	**59:1**	**35:1**
P1	09-Feb-11	Initial	39.7 ± 1.6	15.0 ± 0.3	0.7 ± 0.0	22:1	
		Final	35.9 ± 2.3	11.2 ± 0.5	0.5 ± 0.01	24:1	16:1
P2	11-Feb-11	Initial	43.8 ± 2.3	17.2 ± 0.7	1.0 ± 0.01	18:1	
		Final	41.5 ± 2.7	16.1 ± 0.5	0.8 ± 0.01	20:1	6:1
P3	15-Feb-11	Initial	37.4 ± 3.0	17.2 ± 0.5	1.8 ± 0.1	9:1	
		Final	39.5 ± 4.2	16.3 ± 0.1	1.7 ± 0.01	9:1	8:1
P4	16-Feb-11	Initial	38.9	17.4	1.8	9:1	
		Final	40.7 ± 3.6	18.4 ± 0.1	1.8 ± 0.1	10:1	*
P5	26-Feb-11	Initial	28.9 ± 7.5	13.3 ± 0.9	0.7 ± 0.1	18:1	
		Final	44.8 ± 1.3	17.5 ± 0.1	0.9 ± 0.0	20:1	28:1
P6	15-Mar-11	Initial	36.9 ± 2.3	15.8 ± 0.5	1.0 ± 0.0	16:1	
		Final	55.7 ± 0.3	22.5 ± 0.2	1.3 ± 0.01	18:1	26:1
P7	19-Mar-11	Initial	36.7 ± 3.3	14.2 ± 0.4	0.9 ± 0.0	15:1	
		Final	39.3 ± 1.4	16.4 ± 0.6	0.9 ± 0.01	19:1	*

Table 3.6. Summary of published phytoplankton growth and grazing mortality rates calculated from dilution experiments in the Southern Ocean. μ = phytoplankton growth rate, g = grazing mortality rate, P_i and P_p are the potential microzooplankton grazing pressure on initial phytoplankton stock and primary production, respectively. NR = not reported, NS = not significant.

Location	Sampling month	μ (d^{-1})	g (d^{-1})	P_i (%)	P_p (%)	Reference
Western Antarctic Peninsula	Jan. – Mar.	NS – 0.99	NS – 0.56	NS – 43	NS – 116	This study
Bellingshausen Sea	Nov. – Dec.	NR	0.03 – 0.52	3 – 40	NR	Burkill et al. 1995
Southern Ocean, 0° longitude	Jan. – Feb.	0.24 – 1.68	0.04 – 0.25	4 – 22	9 – 46	Froneman and Perissinotto 1996a
Southern Ocean, 0° longitude	June – July	0.45 – 1.48	0.28 – 0.72	25 – 51	56 – 83	Froneman and Perissinotto 1996a
Southern Ocean, 0° longitude	June – July	0.17 – 1.87	0.02 – 0.58	2 – 44	9 – 61	Froneman and Perissinotto 1996b
King George Island, Antarctic Peninsula	Dec. – Feb.	NS – 1.16	NS – 0.29	NR	0 – 333	Tsuda and Kawaguchi 1997
Indo-Pacific sector of Southern Ocean	Dec. – Jan.	NS – 0.66	0.01 – 0.69	NR	0 - 105	Tsuda and Kawaguchi 1997
Ross Sea	Oct. – Dec.	NR	NS – 0.26	NR	NR	Caron et al. 2000
Ross Sea	Jan. – Feb.	NR	NS – 0.11	NR	NR	Caron et al. 2000
Ross Sea	April	NR	NS	NR	NR	Caron et al. 2000

Table 3.6. continued

Location	Sampling month	μ (d^{-1})	g (d^{-1})	P_i (%)	P_p (%)	Reference
Prydz Bay	Dec. – Jan.	0.11 – 2.60	0.11 – 1.06	10 - 65	34-100	Li et al. 2001
Southern Ocean, 6°E longitude	Dec. – Jan.	NR	0.04 – 0.28	5 – 24	11 – 35	Froneman 2004
Southern Ocean, 140°E longitude	Nov. – Dec.	NR	0.19 – 1.70	NR	NR	Safi et al. 2007
Near Davis Station, East Antarctica	Feb. – Mar.	NS – 0.81	NS – 0.55	NS – 42	NS - 40	Pearce et al. 2008
Near Davis Station, East Antarctica	Apr. – Sept.	NS – 0.44	NS – 1.54	NS -79	NS - 762	Pearce et al. 2008
East Antarctica, 30 – 80°E	Jan. – Feb.	0.28 – 1.81	0.31 – 2.36	30 - 87	16 - 223	Pearce et al. 2010
Sub-Antarctic zone, Near Tasmania	Jan. – Feb.	NS – 1.02	NS – 1.39	NS - 60	NS - 118	Pearce et al. 2011
Kerguelen Islands	Jan. – Feb.	NR	NS – 1.78	NR	NR	Brussaard et al. 2008

Table 3.7. Summary of published bacterial growth and grazing mortality rates in the Southern Ocean. k = bacterial growth rate, g = grazing mortality rate, B_i and B_p are the potential microzooplankton grazing pressure on initial bacterial stock and bacterial production, respectively. NR = not reported, NS = not significant. [a] rates calculated from dilution experiments.

Location	Austral season	k (d^{-1})	g (d^{-1})	B_i (%)	B_p (%)	Reference
Western Antarctic Peninsula	Jan. – Mar.	NS – 0.95	NS – 0.43	NS – 35	NS – 225	This study[a]
McMurdo Sound	Sept. – Jan.	0.14 – 0.68	0.04 – 0.58	NR	28-86	Anderson and Rivkin 2001[a]
East Antarctica, 30 – 80°E	Jan. – Feb.	0.3 – 2.3	0.40 – 2.6	33 – 93	10 – 163	Pearce et al. 2010[a]
Sub-Antarctic zone, Near Tasmania	Jan. – Feb.	0.14 – 0.87	0.20 – 1.03	13 – 58	35 – 215	Pearce et al. 2011[a]
Gerlache Strait, Antarctic Peninsula	Oct. – Nov.	NS – 0.8	NS	NR	NR	Bird and Karl 1999[a]
Prydz Bay	Dec. – Feb.	0.22 – 0.69	0.03 – 0.13	NR	10 – 35	Leakey et al. 1996
Antarctic Confluence	Nov. – Dec.	0.44	0.22 – 0.44	NR	50 – 100	Kuparinen and Bjørnsen 1992
McMurdo Sound	Nov. – Jan.	NS – 0.26	NS – 0.06	NR	<0.5 – 5	Moisan et al. 1991, Putt et al. 1991

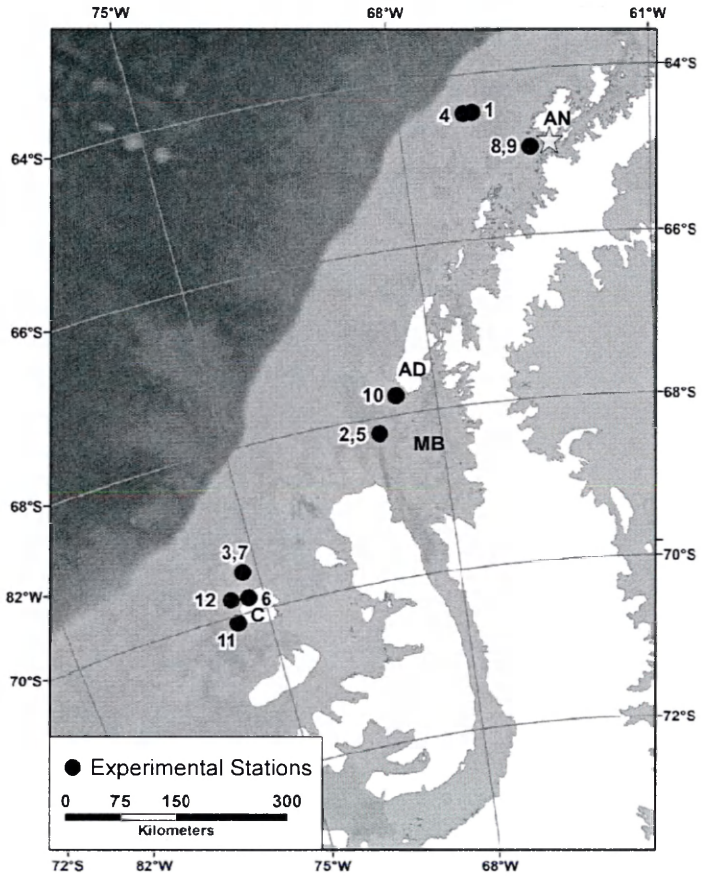


Figure 3.1. Map of the Palmer Antarctica Long-Term Ecological Research study region where dilution experiments were conducted in January 2009 (Experiments 1-3), 2010 (Experiments 4-7) and 2011 (Experiments 8-12). Stations are labeled with Experiment #, multiple labels (e.g., 8,9) indicate more than one experiment conducted at that site. The star marks the location where dilution experiments were conducted near Palmer Station in February – March 2011. AN: Anvers Island, AD: Adelaide Island, MB: Marguerite Bay, C: Charcot Island. Light gray indicates the continental shelf which is about 200 km wide and averages 430 m in depth. The light/dark gray interface indicates the shelf break to waters ~3000 m deep.

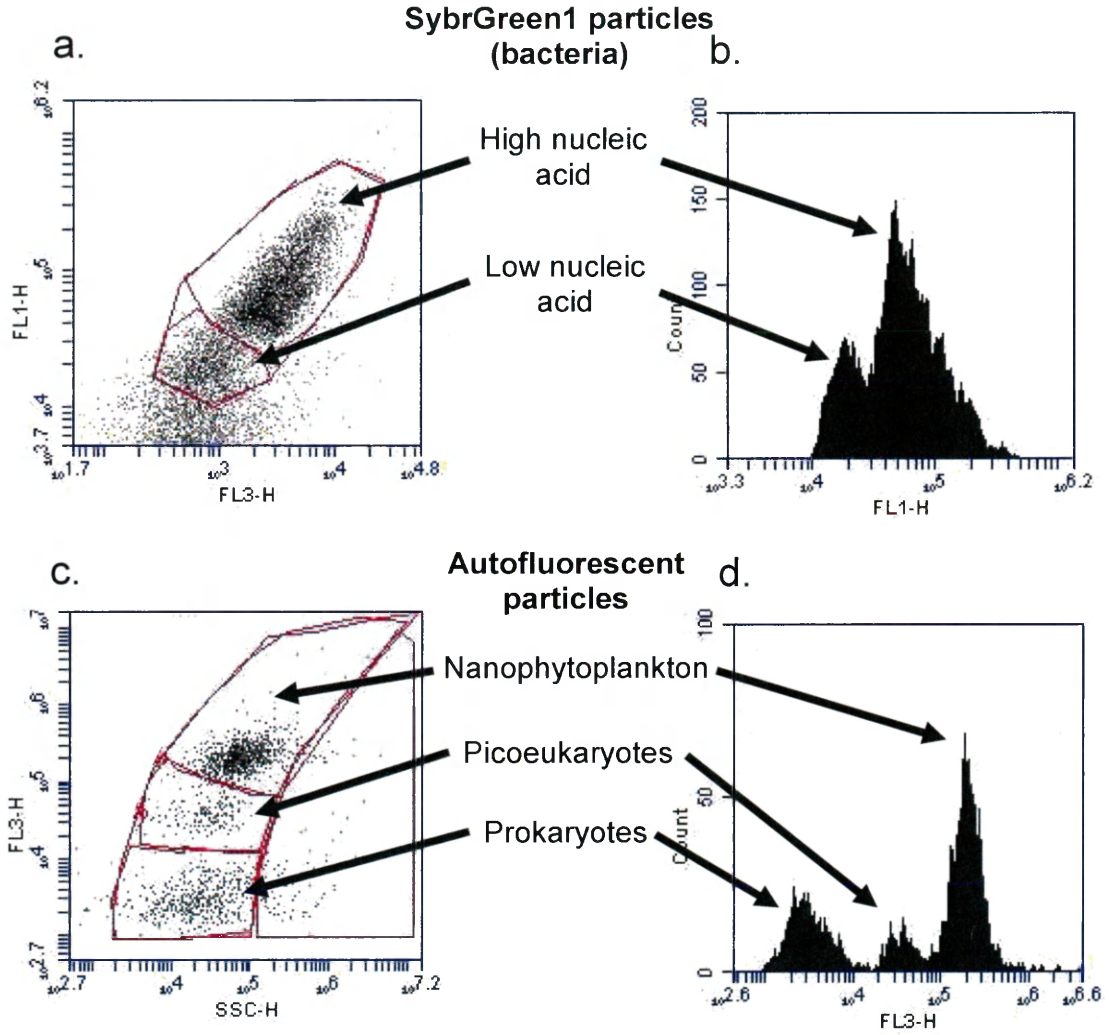


Figure 3.2. Examples of flow cytometric histograms generated with CFlow Plus Software to distinguish between different populations of SybrGreen1 stained bacteria (a,b) and auto fluorescent particles (c,d). (a) Red fluorescence versus green fluorescence scatter plot, (b) green fluorescence histogram, (c) side scatter versus auto fluorescence scatter plot, and (d) auto fluorescence histogram. Size fractions: Nano = Nanophytoplankton (approx. 2 – 20 µm), Pico = Picoeukaryotes (approx. 1 – 2 µm). Prokaryotes were not analyzed in these experiments.

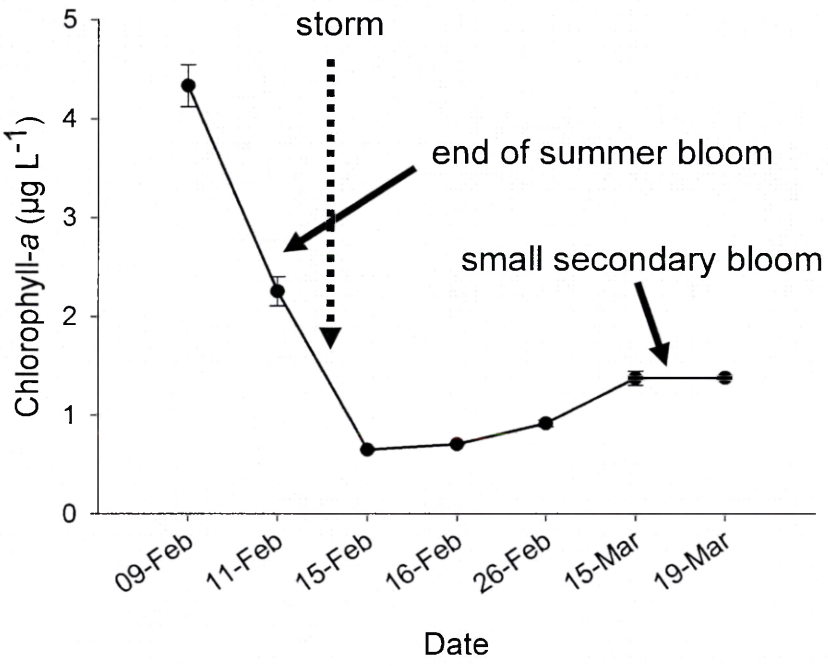


Figure 3.3. Chlorophyll-*a* concentrations from whole water treatments (water screened through 200 µm mesh) at the initial time points from dilution experiments conducted in coastal waters near Palmer Station (64.78°S, 64.04°W) in 2011. Water collected at 5 or 10m. A strong storm with wind gusts up to 60 knots occurred between the experiments on 11-Feb and 15-Feb. Error bars are standard errors, n = 2.

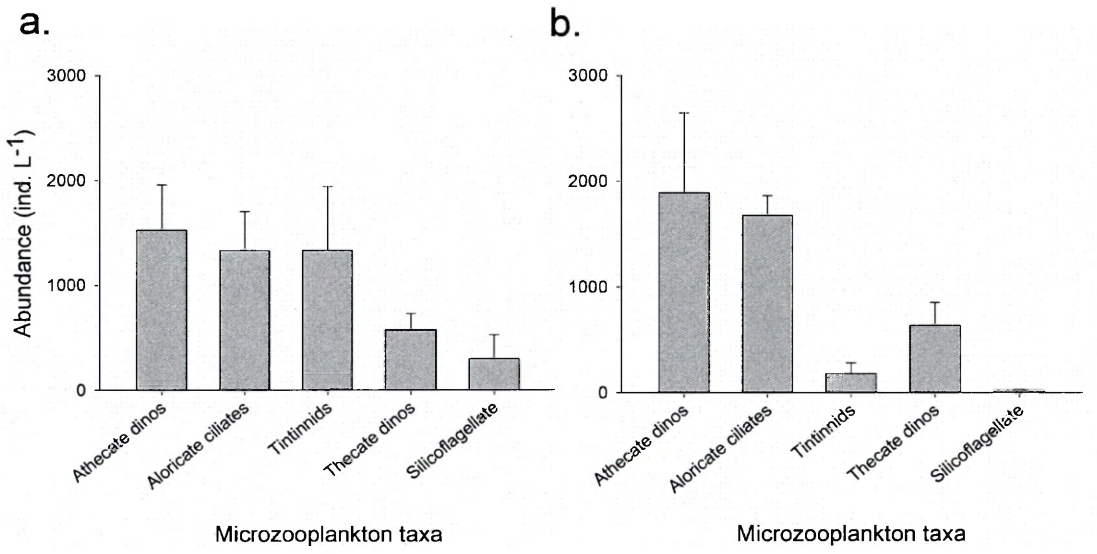


Figure 3.4. Initial microzooplankton abundance in dilution experiments. Data from microscope counts of samples taken from the initial time points of dilution experiments done (a) in January 2010 and 2011 (n=8), and (b) at Palmer Station in February – March 2011 (n = 7). Error bars are standard errors. For Palmer Station (b), one outlier was omitted from the aloricate ciliate average: 39,080 ind. L^{-1} in Experiment P5.

Figure 3.5. (a) Phytoplankton growth and grazing mortality rates, and (b) the percent of primary production removed in dilution experiments conducted at Palmer Station in February – March 2011. Size fractions: Total = as measured by chl-*a* extraction (filtration on GF/F filters, nominal pore size 0.7 μm); Nano = Nanophytoplankton (approx. 2 – 20 μm) and Pico = Picoeukaryotes (approx. 0.5 – 2 μm) size fractions were determined using flow cytometry. One experiment per date. Experiments with no grazing/mortality rates on any phytoplankton size fraction significantly different than zero were omitted (see Table 3.2 for all results). A dash (–) indicates there were no grazing/mortality rates significantly different than zero on a specific phytoplankton size fraction. Arrow indicates occurrence of a strong storm with wind gusts up to 60 knots.

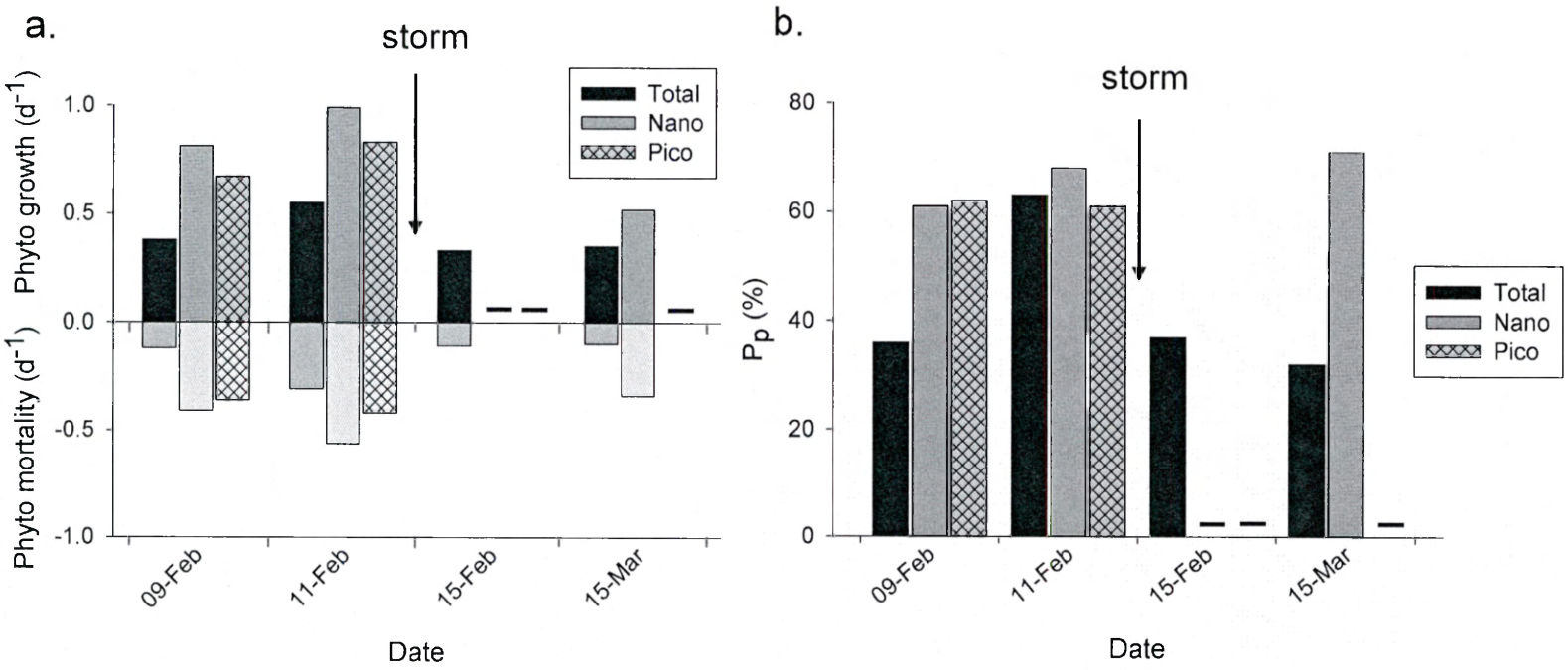

a.
storm
Total
Nano
Pico
Phyto growth (d-1)
Phyto mortality (d-1)
1.0
0.5
0.0
-0.5
-1.0
09-Feb
11-Feb
15-Feb
15-Mar
Date
b.
storm
Pp (%)
80
60
40
20
0
Total
Nano
Pico
09-Feb
11-Feb
15-Feb
15-Mar
Date

Figure 3.6. (a) Bacterial growth and grazing mortality rates, and (b) the percent of bacterial production removed in dilution experiments conducted in January 2010 (Stations 4-6) and 2011 (Stations 8-12). High NA = high nucleic acid content bacteria, Low NA = low nucleic acid content bacteria. For information on 'Total' bacteria, see Table 3.3. Experiments with no grazing/mortality rates on any bacterial type significantly different than zero were omitted (see Table 3.3 for all results). A dash (–) indicates there were no grazing/mortality rates significantly different than zero on a specific bacterial type. See Figure 3.1 and Table 3.1 for experiment locations.

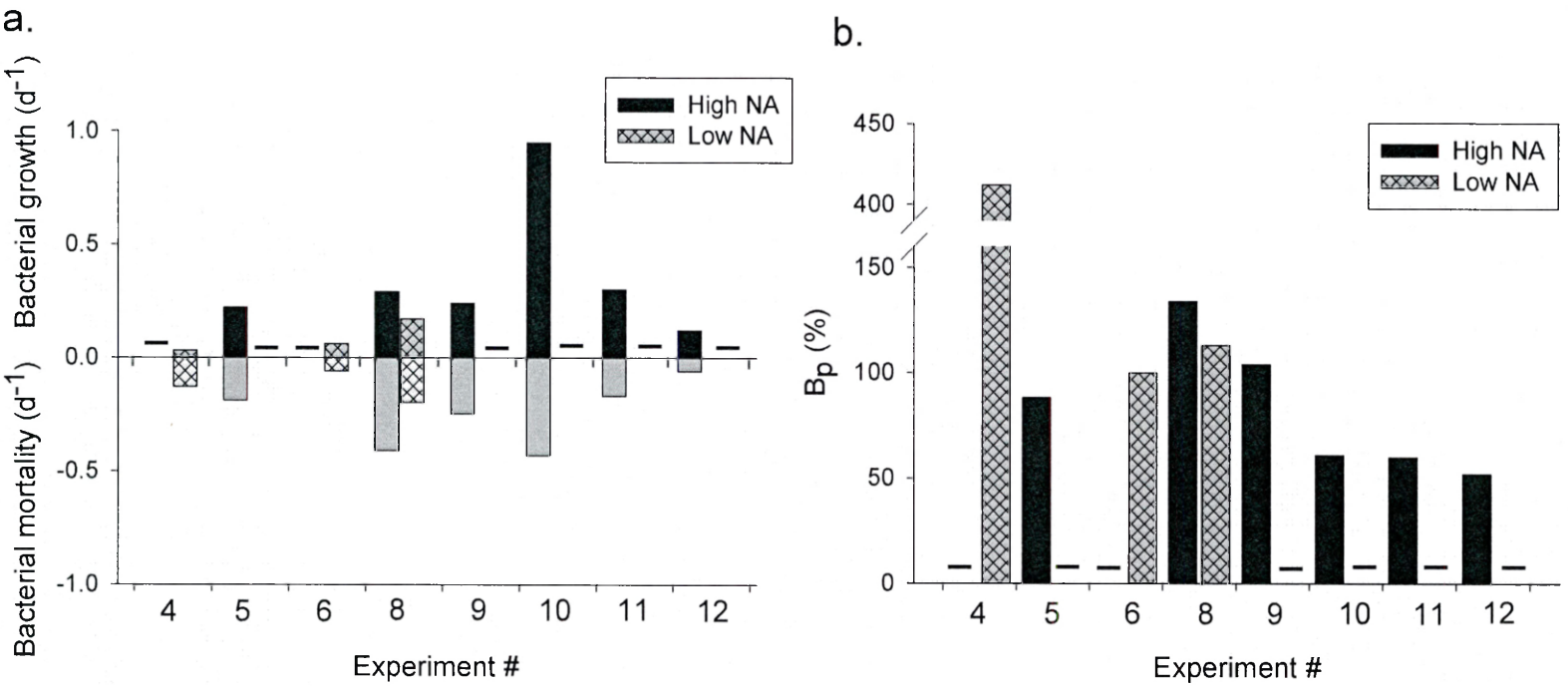

a.
Bacterial growth (d^{-1})
Bacterial mortality (d^{-1})
1.0
0.5
0.0
-0.5
-1.0
High NA
Low NA
4
5
6
8
9
10
11
12
Experiment #
b.
B_p (%)
450
400
150
100
50
0
High NA
Low NA
4
5
6
8
9
10
11
12
Experiment #

Figure 3.7. (a) Bacterial growth and grazing mortality rates, and (b) the percent of bacterial production removed in dilution experiments performed at Palmer Station in February – March 2011. High NA = high nucleic acid content bacteria, Low NA = low nucleic acid content bacteria. For information on 'Total' bacteria, see Table 3.4. One experiment per date. Experiments with no grazing/mortality rates on any bacterial type significantly different than zero were omitted (see Table 3.4 for all results). A dash (–) indicates there were no grazing/mortality rates significantly different than zero on a specific bacterial type. Arrow indicates occurrence of a strong storm with wind gusts up to 60 knots.

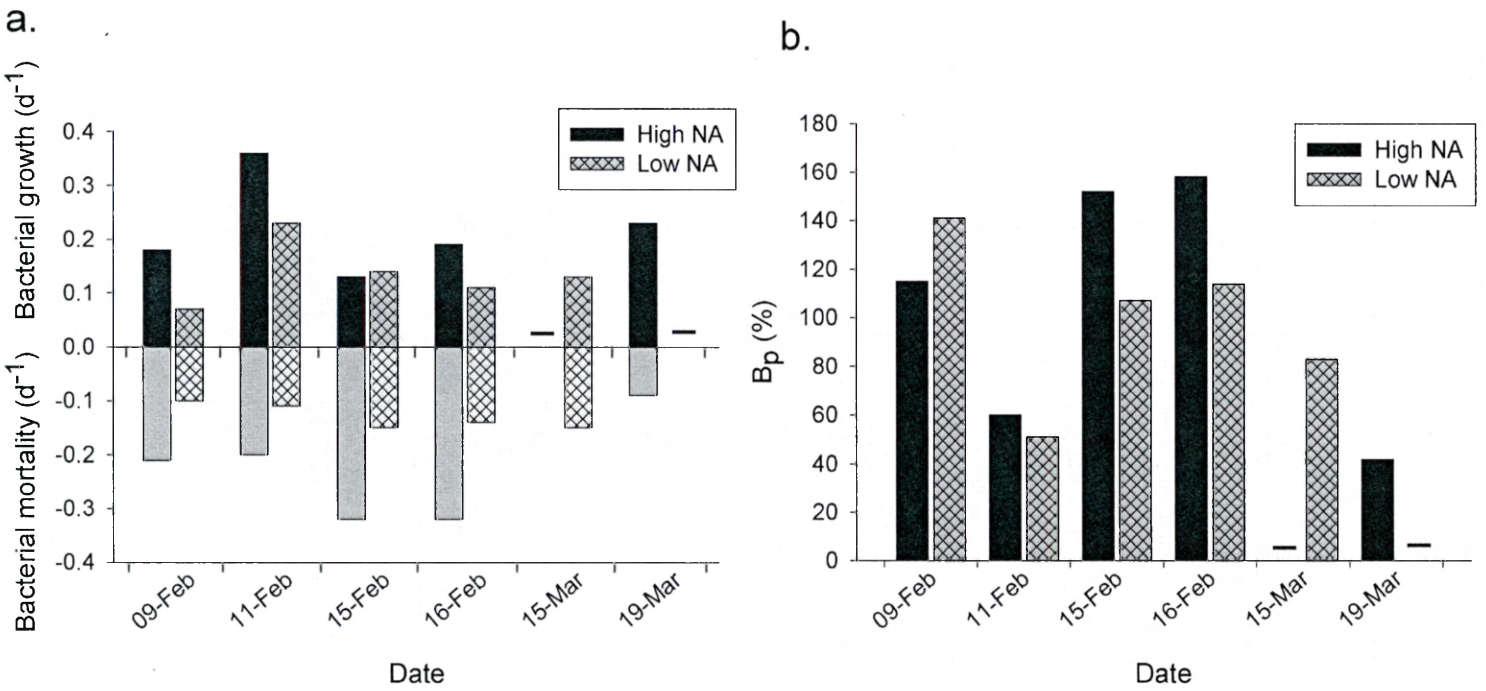

a.
Bacterial mortality (d-1) Bacterial growth (d-1)
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
-0.4
High NA
Low NA
09-Feb
11-Feb
15-Feb
16-Feb
15-Mar
19-Mar
Date
b.
Bp (%)
180
160
140
120
100
80
60
40
20
0
High NA
Low NA
09-Feb
11-Feb
15-Feb
16-Feb
15-Mar
19-Mar
Date

Figure 3.8. Relationship between (a) phytoplankton growth vs. grazing mortality, and (b) bacterial growth vs. grazing mortality. Data from dilution experiments done on the annual LTER cruises in January 2009, 2010 and 2011, and at Palmer Station in February – March 2011. Each point represents growth and mortality rates significantly different than zero on different (a) phytoplankton size fractions, Total = as measured by chl-*a* extraction (filtration on GF/F filters, nominal pore size 0.7 µm); Nano = Nanophytoplankton (approx. 2 – 20 µm) and Pico = Picoeukaryotes (approx. 0.5 – 2 µm); size fractions were determined using flow cytometry, and (b) bacterial types (High NA = high nucleic acid content bacteria, Low NA = low nucleic acid content bacteria) determined using flow cytometry. Linear regression equations: (a) $y = 0.50x + 0.02$ ($p<0.001$. $r^2 = 0.79$), and (b) $y = 0.40x + 0.11$ ($p = 0.003$, $r^2 = 0.42$).

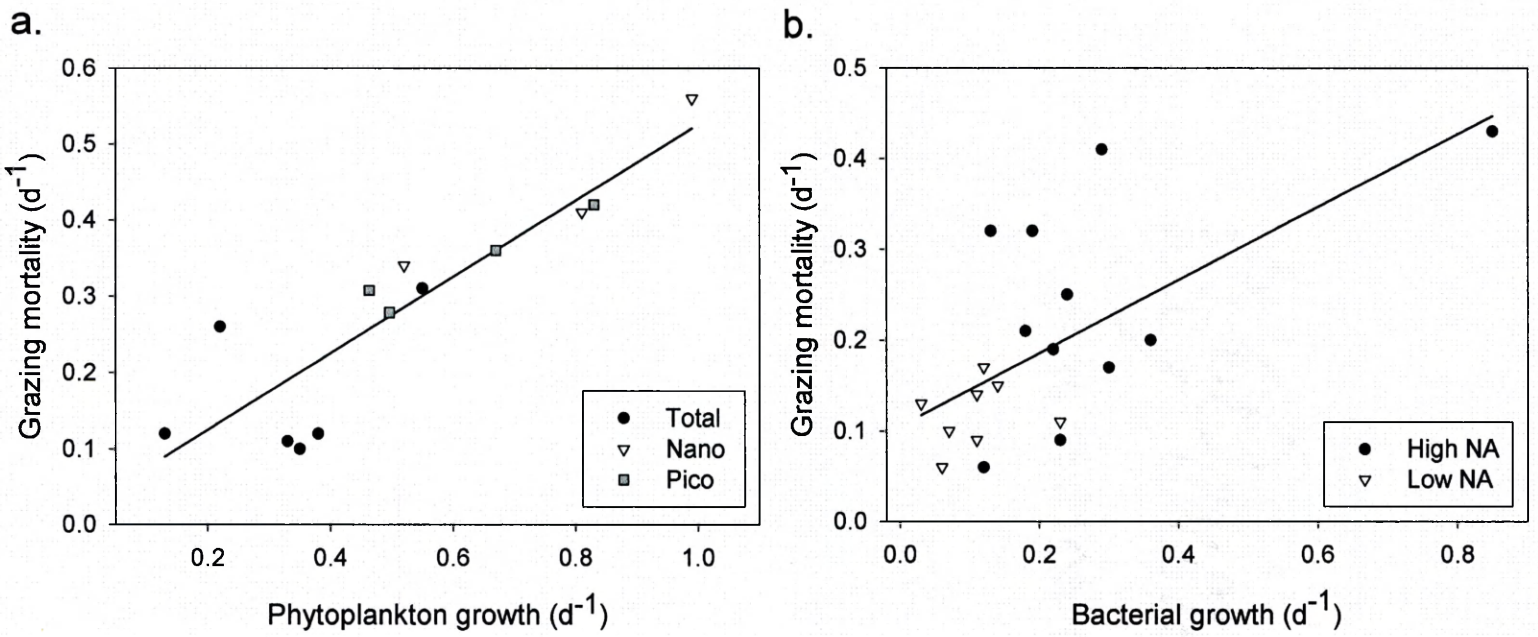
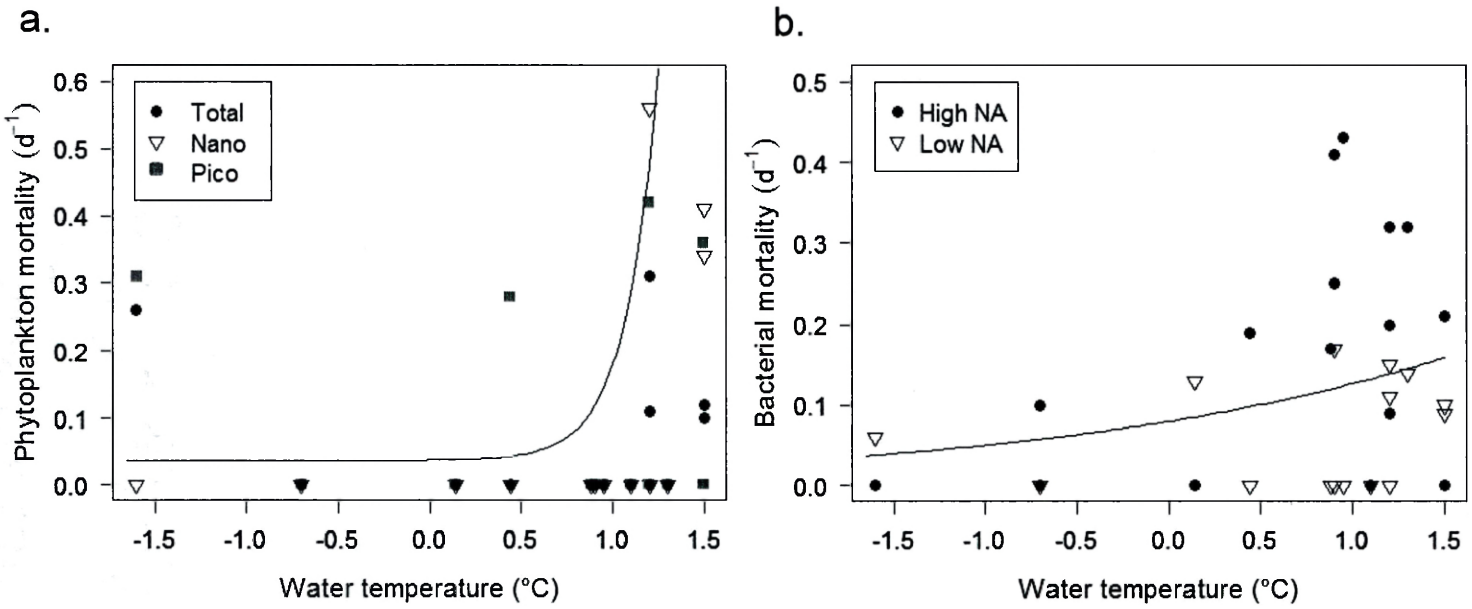

a.
Grazing mortality (d-1)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.2
0.4
0.6
0.8
1.0
Phytoplankton growth (d-1)
Total
Nano
Pico
b.
Grazing mortality (d-1)
0.0
0.1
0.2
0.3
0.4
0.5
0.0
0.2
0.4
0.6
0.8
Bacterial growth (d-1)
High NA
Low NA

Figure 3.9. (a) Phytoplankton grazing mortality, and (b) bacterial grazing mortality as a function of water temperature. Data from dilution experiments performed in January 2010 and 2011, and at Palmer Station in February – March 2011. Each point represents grazing mortality rates (non-significant rates = 0) on different (a) phytoplankton size fractions, Total = as measured by chl-*a* extraction (filtration on GF/F filters); Nano = Nanophytoplankton (approx. 2 – 20 μm) and Pico = Picoeukaryotes (approx. 0.5 – 2 μm) size fractions were determined using flow cytometry, and (b) bacterial types (High NA = high nucleic acid content bacteria, Low NA = low nucleic acid content bacteria) determined using flow cytometry. Each experiment has (a) three, and (b) two corresponding mortality rates. Water temperature was measured by CTD at the location where the water was taken for each experiment. Best-fit equations: (a) $y = 0.03 + 0.0005e^{5.6x}$ ($r^2 = 0.21$, $p = 0.03$, curve excludes the Total size fraction, as Nano and Pico constitute most of Total with the exception of large diatoms), (b) $y = 0.08e^{0.46x}$ ($r^2 = 0.10$, $p = 0.08$). Best-fit equations (data not shown) for (i) phytoplankton growth vs. temperature, $y = 0.006 + 0.0002e^{5.2x}$ ($r^2 = 0.22$, $p = 0.03$), and (ii) bacterial growth vs. temperature, $y = 0.09e^{0.42x}$ ($r^2 = 0.06$, $p = 0.2$). See Tables 3.1 – 3.4 for values.


a.
Phytoplankton mortality (d^{-1})
0.0
0.1
0.2
0.3
0.4
0.5
0.6
Total
Nano
Pico
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
Water temperature (°C)
b.
Bacterial mortality (d^{-1})
0.0
0.1
0.2
0.3
0.4
0.5
High NA
Low NA
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
Water temperature (°C)

CHAPTER 4

Summary and Concluding Remarks

A complete understanding of the marine food web along the Western Antarctic Peninsula (WAP) is essential to understanding how ecosystems in this region will be affected by climate change. This region is experiencing extremely rapid rates of warming (Smith et al. 1996, Vaughan et al. 2003, Ducklow et al. 2012), and declines in sea ice (Stammerjohn et al. 2008) are causing fundamental changes in marine food webs (Schofield et al. 2010). Many components of these food webs (e.g., bacteria, phytoplankton, zooplankton, penguins) have been studied extensively (Clarke et al. 2008, Ross et al. 2008, Erdmann et al. 2011, Ducklow et al. 2012), but microzooplankton have previously been largely overlooked. My research is the first comprehensive study to quantify microzooplankton community structure (Chapter 2) and grazing impact on phytoplankton and bacteria (Chapter 3) along the WAP.

Climate warming is differentially affecting the WAP, creating a "climate gradient" with a warmer sub-Antarctic climate invading the north while the south remains the typical cold, dry Antarctic climate (Smith et al. 1999, Ducklow et al. 2012). Phytoplankton biomass is declining in the north while increasing in the south, and southern assemblages are dominated by diatoms and larger phytoplankton (Montes-Hugo et al. 2009). My research is the first evidence suggesting microzooplankton may also be adjusting to climate trends, as we found generally higher microzooplankton biomass in the south compared to the north, and abundance and biomass of particular taxa such as large tintinnids and thecate dinoflagellates (>60 μm) higher in the south. We cannot say with certainty that this distribution was different before the WAP had warmed substantially, but using the climate gradient is a way to look back in time to when conditions in the north in the past may have been more like they are in the present south.

Microzooplankton biomass was positively correlated with chlorophyll-*a* and particulate organic carbon (POC) in this study, and microzooplankton distribution was also likely influenced by sea ice dynamics, as well as other factors (e.g., water masses, predation pressure). In addition, biomass of some microzooplankton taxa was high near Marguerite Bay, which is known to be a biological hot spot from primary producers to penguins (Hofmann et al. 2002, Ashijan et al. 2004, Vernet et al. 2008, Friedlaender et al. 2011).

Phytoplankton mortality rates were generally lower than averages reported in tropical and temperate habitats (Calbet and Landry 2004) and phytoplankton growth always exceeded microzooplankton grazing. In some experiments phytoplankton growth and mortality rates were not detectable. Other studies have also reported moderate phytoplankton mortality rates in the Antarctic Peninsula region (Burkill et al. 1995, Tsuda and Kawaguchi 1997), and phytoplankton grazing mortality rates not significantly different than zero in a large proportion of experiments conducted in the Ross Sea (Caron et al. 2000). On the other hand, Pearce et al. (2008, 2010) concluded that microzooplankton could remove >100% of primary production at certain times and were key to controlling phytoplankton growth in East Antarctica. Therefore, microzooplankton can exert considerable grazing pressure on phytoplankton at certain times, but during the most productive season along the WAP, microzooplankton did not exert enough grazing pressure on phytoplankton to control large blooms. Temperature could have an effect on microzooplankton grazing rates (Caron et al. 2000, Rose and Caron 2007), but other factors (e.g., presence of large diatoms) likely contributed to the moderate phytoplankton mortality rates in this study.

Microzooplankton exerted higher grazing control on bacteria compared to phytoplankton, and this high grazing pressure could explain the relatively constant bacterial abundances along the WAP. In general, microzooplankton selectively grazed the more actively growing bacterial cells, as well as the smaller, faster-growing phytoplankton size classes. A strong storm at Palmer Station might have affected the microbial food web; data from these experiments indicated a shift from relatively higher grazing on phytoplankton to low grazing rates after the storm, while grazing pressure on bacteria increased after the storm.

With a shift to smaller phytoplankton cells in the northern WAP (Montes-Hugo et al. 2009), one would expect a greater fraction of phytoplankton carbon to be consumed by microzooplankton, a group of grazers that can efficiently consume small cells (Stoecker et al. 1981, Tillmann 2004). Whereas in the south where larger phytoplankton cells often dominated phytoplankton assemblages, a substantial fraction of phytoplankton carbon would be consumed directly by macrozooplankton (e.g. krill). In this study, microzooplankton selectively grazed small phytoplankton size classes, indicating their overall grazing pressure should be higher in the north where small phytoplankton cells dominate. However, microzooplankton grazing rates (when significant) in the north and south were comparable (see Tables 3.1 and 3.2), although microzooplankton removed more Total primary production in the south (89 – 116%, n = 2) compared to the north (32 – 63%, n = 4). Also, Bernard et al. (2012) did not find distinct differences in the primary production removed by macrozooplankton between regions; differences were more related to abundance of *Salpa thompsoni* rather than latitude. Therefore, micro- and

macrozooplankton grazing impact are more complicated than a simple size structure analysis can predict.

My research is the first to describe both the microzooplankton taxonomic distribution and grazing impact in the Palmer Antarctica Long-Term Ecological Research study region.This study contributes valuable information (e.g., microzooplankton biomass estimates, microzooplankton grazing rates) to studies modeling the flow of carbon through the WAP food web (Sailley et al. in prep) and provides a reference point for future research to investigate the impact of climate change on the WAP food web. Further research is needed to determine seasonal variation in microzooplankton distribution and grazing impact, and to clarify the effect of temperature and other factors that affect microzooplankton grazing rates. My research was conducted in the inshore and coastal waters over the continental shelf and future studies should determine microzooplankton grazing impact in oceanic waters past the shelf break to compare with the very high grazing impact of macrozooplankton (particularly salps) on primary productivity. I also did not address the distributions or grazing impact of sarcodines (Radiolaria, Acantharia, Foraminifera), which are important components of Southern Ocean pelagic food webs (Gowing 1989, Klaas 2001), and necessary for a complete understanding of microzooplankton trophic roles. Finally, as microzooplankton are an important component of meso- and macrozooplankton diets in the Southern Ocean (Atkinson and Snÿder 1997, Lonsdale et al. 2000, Schmidt et al. 2006), and of larval fish (Stoecker and Capuzzo 1990, Fukami et al. 1999), more research is also needed to determine top-down controls on microzooplankton. These future research directions will lead to a more comprehensive understanding of the diverse role microzooplankton play in

the pelagic food web, which will inform biogeochemical models and more accurately predict the sinks, sources, and flows of carbon through the WAP food web.

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APPENDIX

Appendix 1. Physical and biological data at each location/depth where microzooplankton samples were collected in the Palmer Antarctica Long-Term Ecological Research (PAL LTER) study area in 2010. Grid = PAL LTER station identification (Waters and Smith 1992). Lat = Latitude, Long = Longitude. Temp = water temperature. Chl-*a* = chlorophyll-*a* concentrations determined fluorometrically. POC = particulate organic carbon. A dash (-) indicates data not available. *sample collected at 30 m. Data courtesy of O. Schofield and H. Ducklow and can be found at http://pal.lternet.edu/data/.

Grid	Lat. (°S)	Long. (°W)	Date	Depth (m)	Temp. (°C)	Salinity	Chl-*a* (μg L^{-1})	POC (μg L^{-1})
600.040	64.9	64.4	05-Jan	10	0.56	33.9	1.13	224
				20	0.51	33.9	1.14	220
				100	0.11	34.3	0.07	28.6
600.100	64.6	65.3	06-Jan	10	0.18	33.9	1.29	197
				30	0.18	33.9	1.05	188
				100	-0.80	34.2	0.005	37.8
600.200	64.0	66.9	07-Jan	10	0.24	33.7	0.220	108
				20	0.24	33.7	0.265*	96.6*
				100	-0.75	34.1	0.250	42.3
585.135	64.5	66.2	09-Jan	10	0.56	33.8	0.410	-
				50	0.07	33.8	0.663	-
				100	-1.1	34.2	0.259	-
500.200	64.6	68.3	07-Jan	10	-0.03	33.8	0.335	97.4
				70	-1.5	34.0	0.747	59.9
				100	-0.52	34.2	0.331	27.5

Appendix 1. continued

Grid	Lat. (°S)	Long. (°W)	Date	Depth (m)	Temp. (°C)	Salinity	Chl-*a* (µg L^{-1})	POC (µg L^{-1})
400.040	66.3	67.3	11-Jan	10	0.95	33.8	3.06	301
				30	0.95	33.8	3.17	311
				100	-0.50	34.2	0.056	18.9
400.100	65.9	68.3	11-Jan	10	0.46	33.7	1.66	247
				45	0.24	33.7	3.46	212
				100	-0.51	34.3	0.180	23.8
400.160	65.5	69.2	12-Jan	10	0.21	33.7	0.671	144
				50	-0.13	33.8	0.243	131
				100	-0.86	34.2	0.026	30.5
200.-040	68.0	69.3	24-Jan	20	0.85	33.7	2.21	325
				100	-1.2	34.0	0.092	31.0
200.040	67.5	70.6	14-Jan	10	-0.11	33.7	1.72	199
				35	-0.16	33.7	1.25	185
				100	-1.6	34.1	0.160	28.9
200.100	67.1	71.5	14-Jan	10	-0.08	33.8	0.771	189
				35	-0.08	33.8	1.55	180
				100	-1.2	34.2	0.158	18.3
200.160	66.7	72.5	16-Jan	10	-0.07	33.6	1.17	195
				40	-0.07	33.6	1.10	229
				100	-0.94	34.1	0.145	31.1
167.-033	68.2	70.0	18-Jan	10	0.60	33.7	2.56	-
				35	0.44	33.7	0.102*	-
				100	-1.3	34.0	0.002	-

Appendix 1. continued

Grid	Lat. (°S)	Long. (°W)	Date	Depth (m)	Temp. (°C)	Salinity	Chl-*a* (μg L^{-1})	POC (μg L^{-1})
100.000	68.4	71.7	22-Jan	10	0.63	33.6	3.49	300
				18	0.63	33.6	3.44	291
				95	-0.94	34.1	0.350	25.9
100.040	68.1	72.3	16-Jan	10	-0.36	33.8	1.38	177
				40	-0.40	33.8	1.43	175
				100	-1.4	34.1	0.107	28.4
100.100	67.7	73.3	16-Jan	10	0.22	33.8	0.778	122
				35	0.22	33.7	0.656	-
				100	-1.78	34.1	0.306	54.7
000.000	69.0	75.6	21-Jan	12	-0.35	33.3	2.98	465
				35	-0.37	33.4	4.23	439
				100	-1.5	33.8	1.83	51.3
000.040	68.7	74.2	21-Jan	15	-0.33	33.7	1.63	249
				100	-1.5	34.0	0.177	26.0
000.120	68.1	75.4	21-Jan	10	0.19	33.8	0.613	139
				45	-0.18	33.8	1.38	118
				100	-1.8	34.1	0.230	31.4
-100.000	69.5	75.5	27-Jan	12	-0.71	33.1	8.51	598
				30	-1.2	33.4	1.16	97.2
				100	-1.5	33.8	0.121	32.5
-121.-024	69.8	75.5	26-Jan	10	-1.4	33.1	2.35	-
				20	-1.6	33.3	1.81	-
				100	-1.6	33.8	0.103	-

Appendix 2. Physical and biological data collected at each location/depth where microzooplankton samples were collected in the Palmer Antarctica Long-Term Ecological Research (PAL LTER) study area in 2011. Grid = PAL LTER station identification (Waters and Smith 1992). Lat = Latitude, Long = Longitude. Temp = water temperature. Chl-*a* = chlorophyll-*a* concentrations determined fluorometrically. POC = particulate organic carbon. A dash (-) indicates data not available. Data courtesy of O. Schofield and H. Ducklow and can be found at http://pal.lternet.edu/data/.

Grid	Lat. (°S)	Long. (°W)	Date	Depth (m)	Temp. (°C)	Salinity	Chl-*a* (μg L^{-1})	POC (μg L^{-1})
600.040	64.9	64.4	11-Jan	10	1.2	33.5	1.62	164
				20	0.20	33.6	0.540	68.6
				100	0.25	34.2	0.063	21.9
600.100	65.3	64.6	07-Jan	10	0.66	33.7	0.777	77.1
				25	0.84	33.8	0.680	130
				100	0.29	34.0	0.298	47.5
600.200	66.9	64.0	07-Jan	12	0.51	33.9	0.175	64.6
				70	-0.17	34.0	0.273	38.2
				100	-0.16	34.1	0.160	37.1
500.120	67.1	65.1	09-Jan	10	0.95	34.0	1.01	134
				40	0.45	34.0	1.08	117
				100	-0.27	34.0	0.413	50.3
500.200	68.3	64.6	08-Jan	10	0.59	33.9	0.179	64.0
				65	-0.40	34.0	0.237	44.5
				100	0.74	34.4	0.039	29.3

Appendix 2. continued

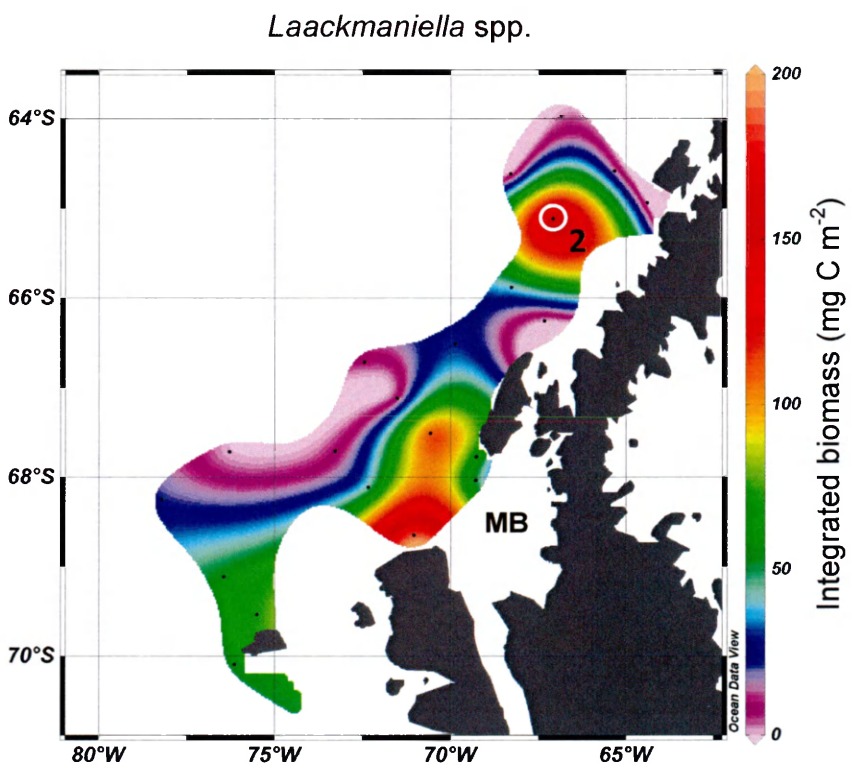
Grid	Lat. (°S)	Long. (°W)	Date	Depth (m)	Temp. (°C)	Salinity	Chl-*a* (μg L^{-1})	POC (μg L^{-1})
400.040	66.3	67.3	13-Jan	5	1.8	33.8	1.83	302
				30	1.2	33.8	2.01	212
				100	-0.36	34.1	0.186	42.3
400.100	65.9	68.3	13-Jan	10	0.93	33.9	1.74	226
				45	0.01	34.0	0.514	83.6
				100	-0.36	34.2	0.039	34.3
300.100	66.5	69.9	14-Jan	10	0.62	33.9	2.07	193
				20	0.57	33.9	1.80	165
				100	-1.2	34.0	0.033	30.1
221.-019	67.8	69.3	18-Jan	10	0.64	33.8	10.8	499
				15	0.29	33.8	11.4	387
				100	-0.80	34.0	0.771	-
200.-040	68.0	69.3	16-Jan	10	1.3	33.3	25.1	1239
				15	0.17	33.4	13.2	-
				100	-1.2	34.0	1.33	91.2
200.040	67.5	70.6	15-Jan	10	2.0	33.5	1.52	436
				40	-0.14	33.8	14.2	714
				110	-1.5	33.9	0.533	52.2
200.100	67.1	71.5	14-Jan	10	0.82	33.9	2.50	219
				25	0.72	33.9	0.518	75.9
				100	-1.2	34.0	0.062	30.2

Appendix 2. continued

Grid	Lat. (°S)	Long. (°W)	Date	Depth (m)	Temp. (°C)	Salinity	Chl-*a* (µg L^{-1})	POC (µg L^{-1})
200.160	66.7	72.5	17-Jan	10	1.0	33.8	5.75	189
				40	0.22	33.8	0.063	105
				100	-0.21	34.2	0.117	31.6
100.-040	68.6	71.0	25-Jan	10	0.53	32.7	6.72	893
				20	0.50	32.8	8.64	723
				100	-1.2	33.9	0.427	21.2
100.040	68.1	72.3	25-Jan	15	1.8	33.9	0.127	323
				25	1.8	33.9	3.65	310
				100	-1.3	34.0	0.294	26.7
100.100	67.7	73.3	25-Jan	10	1.1	33.9	1.38	181
				25	1.1	33.9	0.157	157
				100	-1.3	34.1	0.067	33.6
000.180	67.7	76.3	26-Jan	15	0.95	33.9	0.306	84.1
				55	0.43	33.9	0.309	119
				100	-0.40	34.2	0.128	34.8
-100.000	69.5	75.5	30-Jan	10	0.80	32.9	4.29	895
				30	-0.55	33.3	13.3	892
				100	-1.6	33.8	-	-
-100.060	-69.1	-76.4	27-Jan	10	1.1	33.2	5.30	817
				25	0.48	33.7	5.98	505
				100	-1.3	33.9	0.215	45.6

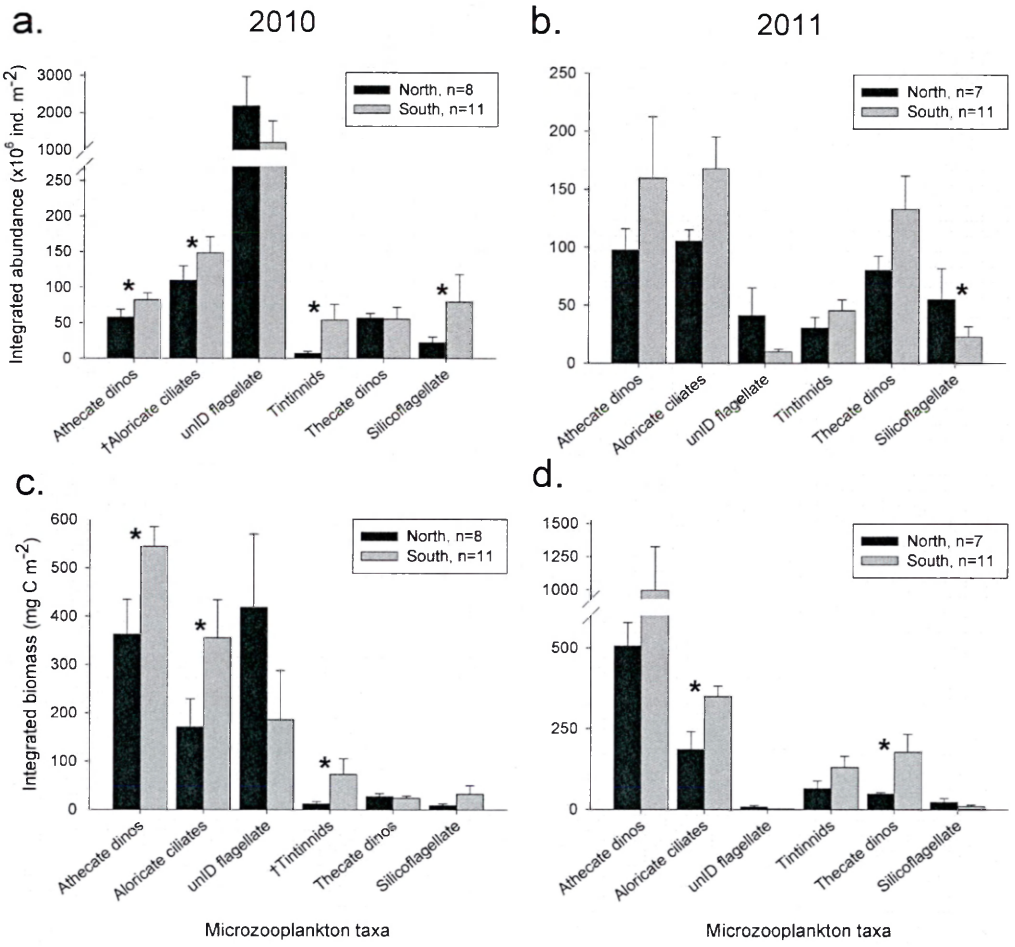
Appendix 2. continued

Grid	Lat. (°S)	Long. (°W)	Date	Depth (m)	Temp. (°C)	Salinity	Chl-*a* (µg L^{-1})	POC (µg L^{-1})
-100.180	68.2	78.2	26-Jan	10	0.90	33.7	0.48	179
				40	-0.23	33.8	1.35	240
				100	-0.84	34.1	-	57.7
-158.-034	70.1	76.1	29-Jan	10	-0.54	32.8	9.55	1320
				20	0.18	33.4	13.5	658
				100	-1.7	33.9	0.484	-



Appendix 3. Integrated biomass (0-100m) of the large tintinnids *Laackmaniella* spp. in 2011. Station 2 is circled (see Figure 2.1). MB: Marguerite Bay.

Appendix 4. Integrated abundance (0-100m) in (a) 2010 and (b) 2011, and integrated biomass (0-100m) in (c) 2010 and (d) 2011 for the major microzooplankton taxa in the northern and southern sub-regions of the Western Antarctic Peninsula, with stations near Marguerite Bay removed (Station 3, and the three stations clustered just south of Adelaide Island at the mouth of Marguerite Bay, see Figure 2.1). Error bars are standard error, asterisks (*) indicates statistically significant ($p<0.05$) differences in abundance and biomass between sub-regions (north vs. south). † indicates a significant interaction between sub-region (north, south) and location (inshore, midshelf, offshore) in the 2-way ANOVA for ciliate abundance and tintinnid biomass in 2010. For statistical analysis, data were transformed using the natural log transformation to fit the normality and homogeneity of variance assumptions of the ANOVA. See Figure 2.1 for map showing division of northern and southern sub-regions.


a.
2010
b.
2011
Integrated abundance (x10^6 ind. m^-2)
North, n=8
South, n=11
North, n=7
South, n=11
Athecate dinos
†Aloricate ciliates
unID flagellate
Tintinnids
Thecate dinos
Silicoflagellate
Aloricate ciliates
c.
d.
Integrated biomass (mg C m^-2)
†Tintinnids
Microzooplankton taxa

Appendix 5. Summary of microzooplankton abundance (ind. L^{-1}) at the initial and final time points in dilution experiments done on the annual LTER oceanographic cruise in January of 2010 and 2011, as well as experiments done at Palmer Station in February – March 2011. See Table 3.1 for additional site information. One sample analyzed for each time point. + = increased 25-100%, ++ = more than doubled, § = decreased 25-50%, §§ = decreased by more than half.

Expt. #	Date	Time point	Athecate dinos	Aloricate ciliates	Tintinnids	Thecate dinos	Silicoflagellate
4	08-Jan-2010	Initial	1230	950	10	600	190
		Final	$220^{\S\S}$	990	10	1030^{+}	$130^{\S}$
5	18-Jan-2010	Initial	1940	760	180	880	20
		Final			Sample lost		
6	26-Jan-2010	Initial	400	720	500	130	20
		Final	580^{+}	$340^{\S}$	$300^{\S}$	360^{++}	40^{++}
7	27-Jan-2010	Initial	1340	2210	4940	480	1890
		Final	2286^{+}	$1160^{\S}$	$1960^{\S}$	594^{+}	2880^{+}
8	11-Jan-2011	Initial	610	480	110	190	40
		Final	2290^{++}	1390^{++}	310^{++}	630^{++}	180^{++}
9	12-Jan-2011	Initial	250	450	90	380	10
		Final	1050^{++}	1160^{++}	280^{++}	1120^{++}	50^{++}
10	18-Jan-2011	Initial	2110	800	2780	390	140
		Final	3775^{+}	$475^{\S}$	3200	1075^{++}	$75^{\S}$
11	29-Jan-2011	Initial	3925	1500	963	1488	13
		Final	7038^{+}	$700^{\S}$	$500^{\S}$	1837	10

Appendix 5. continued

Expt. #	Date	Time point	Athecate dinos	Aloricate ciliates	Tintinnids	Thecate dinos	Silicoflagellate
12	29-Jan-2011	Initial	2325	3525	1275	938	88
		Final	3320^{+}	3006	968	850	$17^{\S}$
P1	09-Feb-2011	Initial	980	2110	140	800	50
		Final	3390^{++}	$960^{\S}$	$50^{\S}$	1320^{+}	$20^{\S}$
P2	11-Feb-2011	Initial	1060	1620	50	180	10
		Final	1080	1910	40	690^{+}	10
P3	15-Feb-2011	Initial	930	2330	60	340	10
		Final	1000	2210	$20^{\S}$	$170^{\S}$	20^{+}
P4	16-Feb-2011	Initial	670	1200	30	100	10
		Final	850^{+}	963	25	$75^{\S}$	13^{+}
P5	26-Feb-2011	Initial	6350	39080	780	1800	30
		Final	$2330^{\S\S}$	$3620^{\S\S}$	$300^{\S\S}$	$610^{\S\S}$	60^{++}
P6	15-Mar-2011	Initial	1420	1610	150	760	10
		Final	$1020^{\S}$	$700^{\S\S}$	$60^{\S\S}$	1200^{+}	40^{++}
P7	19-Mar-2011	Initial	1830	1180	50	470	50
		Final	1788	$325^{\S\S}$	113^{++}	$175^{\S\S}$	138^{++}

VITA

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Born September 24, 1985 in Pittsburgh, PA. Graduated from Plum Senior High School in 2004. Earned a B.S. in Biology from Messiah College in 2008. Worked as a Research Intern with Grace Saba at the Virginia Institute of Marine Science on zooplankton grazing on harmful algal species in the Chesapeake Bay. Was a Teaching and Research Assistant at the Institute for Tropical Marine Ecology in the Commonwealth of Dominica with Dr. Sascha Steiner. Worked as a Research Intern for Dr. Frank Hernandez and Dr. Hugh MacIntyre at the Dauphin Island Sea Lab on the Fisheries Oceanography of Coastal Alabama (FOCAL) project. Entered the M.S. program at the Virginia Institute of Marine Science, College of William and Mary under graduate advisor Dr. Deborah K. Steinberg in 2009.